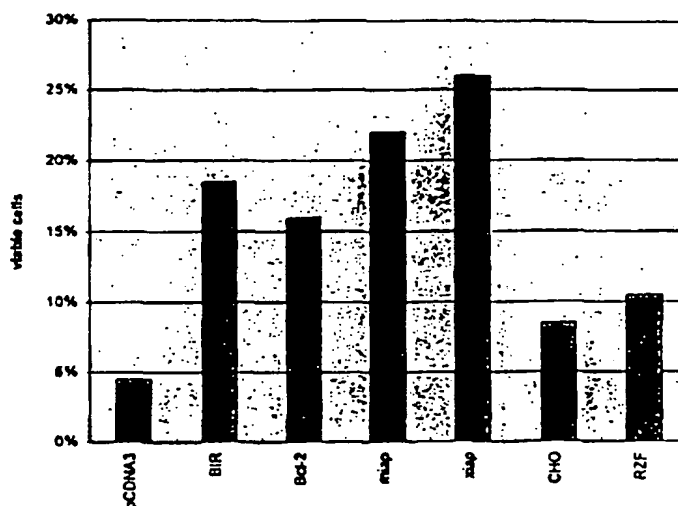




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(54) Title: MAMMALIAN APOPTOSIS INHIBITOR PROTEIN GENE FAMILY, PRIMERS, PROBES AND DETECTION METHODS



BIR = BACULOVIRUS IAP REPEAT
RZF = RING ZINC FINGER

(57) Abstract

Disclosed is substantially pure DNA encoding mammalian IAP polypeptides; substantially pure polypeptides; and methods of using such DNA to express the IAP polypeptides in cells and animals to inhibit apoptosis. Also disclosed are conserved regions characteristic of the IAP family and primers and probes for the identification and isolation of additional IAP genes. In addition, methods for treating diseases and disorders involving apoptosis are provided.

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MAMMALIAN APOPTOSIS INHIBITOR PROTEIN GENE FAMILY, PRIMERS,
PROBES AND DETECTION METHOD

Background of the Invention

5 The invention relates to apoptosis.

 There are two general ways by which cells die.
The most easily recognized way is by necrosis, which is
usually caused by an injury that is severe enough to
disrupt cellular homeostasis. Typically, the cell's
10 osmotic pressure is disturbed and, consequently, the cell
swells and then ruptures. When the cellular contents are
spilled into the surrounding tissue space, an
inflammatory response often ensues.

 The second general way by which cells die is
15 referred to as apoptosis, or programmed cell death.
Apoptosis often occurs so rapidly that it is difficult to
detect. This may help to explain why the involvement of
apoptosis in a wide spectrum of biological processes has
only recently been recognized.

20 The apoptosis pathway has been highly conserved
throughout evolution, and plays a critical role in
embryonic development, viral pathogenesis, cancer,
autoimmune disorders, and neurodegenerative disease. For
example, inappropriate apoptosis may cause or contribute
25 to AIDS, Alzheimer's Disease, Parkinson's Disease,
Amyotrophic Lateral Sclerosis (ALS), retinitis pigmentosa
and other diseases of the retina, myelodysplastic
syndrome (e.g. aplastic anemia), toxin-induced liver
disease, including alcoholism, and ischemic injury
30 (e.g. myocardial infarction, stroke, and reperfusion
injury). Conversely, the failure of an apoptotic
response has been implicated in the development of
cancer, particularly follicular lymphoma, p53-mediated
carcinomas, and hormone-dependent tumors, in autoimmune
35 disorders, such as lupus erythematosus and multiple

- 2 -

sclerosis, and in viral infections, including those associated with herpes virus, poxvirus, and adenovirus.

In patients infected with HIV-1, mature CD4⁺ T lymphocytes respond to stimulation from mitogens or super-antigens by undergoing apoptosis. However, the great majority of these cells are not infected with the virus. Thus, inappropriate antigen-induced apoptosis could be responsible for the destruction of this vital part of the immune system in the early stages of HIV infection.

Baculoviruses encode proteins that are termed inhibitors of apoptosis proteins (IAPs) because they inhibit the apoptosis that would otherwise occur when insect cells are infected by the virus. These proteins are thought to work in a manner that is independent of other viral proteins. The baculovirus IAP genes include sequences encoding a ring zinc finger-like motif (RZF), which is presumed to be directly involved in DNA binding, and two N-terminal domains that consist of a 70 amino acid repeat motif termed a BIR domain (Baculovirus IAP Repeat).

Summary of the Invention

In general, the invention features a substantially pure DNA molecule, such as a genomic, cDNA, or synthetic DNA molecule, that encodes a mammalian IAP polypeptide. This DNA may be incorporated into a vector, into a cell, which may be a mammalian, yeast, or bacterial cell, or into a transgenic animal or embryo thereof. In preferred embodiments, the DNA molecule is a murine gene (e.g., m-xiap, m-hiap-1, or m-hiap-2) or a human gene (e.g., xiap, hiap-1, or hiap-2). In most preferred embodiments the IAP gene is a human IAP gene. In other various preferred embodiments, the cell is a transformed cell. In related aspects, the invention features a transgenic animal

- 3 -

containing a transgene that encodes an IAP polypeptide that is expressed in or delivered to tissue normally susceptible to apoptosis, i.e., to a tissue that may be harmed by either the induction or repression of
5 apoptosis. In yet another aspect, the invention features DNA encoding fragments of IAP polypeptides including the BIR domains and the RZF domains provided herein.

In specific embodiments, the invention features DNA sequences substantially identical to the DNA
10 sequences shown in Figs. 1-6, or fragments thereof. In another aspect, the invention also features RNA which is encoded by the DNA described herein. Preferably, the RNA is mRNA. In another embodiment the RNA is antisense RNA.

In another aspect, the invention features a
15 substantially pure polypeptide having a sequence substantially identical to one of the IAP amino acid sequences shown in Figures 1-6.

In a second aspect, the invention features a substantially pure DNA which includes a promoter capable
20 of expressing the IAP gene in a cell susceptible to apoptosis. In preferred embodiments, the IAP gene is xiap, hiap-1, or hiap-2. Most preferably, the genes are human or mouse genes. The gene encoding hiap-2 may be the full-length gene, as shown in Fig. 3, or a truncated
25 variant, such as a variant having a deletion of the sequence boxed in Fig. 3.

In preferred embodiments, the promoter is the promoter native to an IAP gene. Additionally, transcriptional and translational regulatory regions are,
30 preferably, those native to an IAP gene. In another aspect, the invention provides transgenic cell lines and transgenic animals. The transgenic cells of the invention are preferably cells that are altered in their apoptotic response. In preferred embodiments, the
35 transgenic cell is a fibroblast, neuronal cell, a

- 4 -

lymphocyte cell, a glial cell, an embryonic stem cell, or an insect cell. Most preferably, the neuron is a motor neuron and the lymphocyte is a CD4⁺ T cell.

In another aspect, the invention features a method
5 of inhibiting apoptosis that involves producing a transgenic cell having a transgene encoding an IAP polypeptide. The transgene is integrated into the genome of the cell in a way that allows for expression. Furthermore, the level of expression in the cell is
10 sufficient to inhibit apoptosis.

In a related aspect, the invention features a transgenic animal, preferably a mammal, more preferably a rodent, and most preferably a mouse, having either increased copies of at least one IAP gene inserted into
15 the genome (mutant or wild-type), or a knockout of at least one IAP gene in the genome. The transgenic animals will express either an increased or a decreased amount of IAP polypeptide, depending on the construct used and the nature of the genomic alteration. For example, utilizing
20 a nucleic acid molecule that encodes all or part of an IAP to engineer a knockout mutation in an IAP gene would generate an animal with decreased expression of either all or part of the corresponding IAP polypeptide. In contrast, inserting exogenous copies of all or part of an
25 IAP gene into the genome, preferably under the control of active regulatory and promoter elements, would lead to increased expression or the corresponding IAP polypeptide.

In another aspect, the invention features a method
30 of detecting an IAP gene in a cell by contacting the IAP gene, or a portion thereof (which is greater than 9 nucleotides, and preferably greater than 18 nucleotides in length), with a preparation of genomic DNA from the cell. The IAP gene and the genomic DNA are brought into
35 contact under conditions that allow for hybridization

- 5 -

(and therefore, detection) of DNA sequences in the cell that are at least 50% identical to the DNA encoding HIAP-1, HIAP-2, or XIAP polypeptides.

In another aspect, the invention features a method
5 of producing an IAP polypeptide. This method involves providing a cell with DNA encoding all or part of an IAP polypeptide (which is positioned for expression in the cell), culturing the cell under conditions that allow for expression of the DNA, and isolating the IAP polypeptide.
10 In preferred embodiments, the IAP polypeptide is expressed by DNA that is under the control of a constitutive or inducible promotor. As described herein, the promotor may be a heterologous promotor.

In another aspect, the invention features
15 substantially pure mammalian IAP polypeptide. Preferably, the polypeptide includes an amino acid sequence that is substantially identical to all, or to a fragment of, the amino acid sequence shown in any one of Figs. 1-4. Most preferably, the polypeptide is the XIAP,
20 HIAP-1, HIAP-2, M-XIAP, M-HIAP-1, or M-HIAP-2 polypeptide. Fragments including one or more BIR domains (to the exclusion of the RZF), the RZF domain (to the exclusion of the BIR domains), and a RZF domain with at least one BIR domain, as provided herein, are also a part
25 of the invention.

In another aspect, the invention features a recombinant mammalian polypeptide that is capable of modulating apoptosis. The polypeptide may include at least a ring zinc finger domain and a BIR domain as
30 defined herein. In preferred embodiments, the invention features (a) a substantially pure polypeptide, and (b) an oligonucleotide encoding the polypeptide. In instances where the polypeptide includes a ring zinc finger domain, the ring zinc finger domain will have a sequence
35 conforming to: Glu-Xaal-Xaal-Xaal- Xaal-Xaal-Xaal-Xaa2-

- 6 -

Xaal-Xaal-Xaal-Cys-Lys-Xaa3-Cys-Met-Xaal-Xaal-Xaal-Xaal-Xaal-Xaa3-Xaal-Phe-Xaal-Pro-Cys-Gly-His-Xaal-Xaal-Xaal-Cys-Xaal-Xaal-Cys-Ala-Xaal-Xaal-Xaal-Xaal-Xaal-Cys-Pro-Xaal-Cys, where Xaal is any amino acid, Xaa2 is Glu or
 5 Asp, Xaa3 is Val or Ile (SEQ ID NO:1); and where the polypeptide includes at least one BIR domain, the BIR domain will have a sequence conforming to: Xaal-Xaal-Xaal-Arg-Leu-Xaal-Thr-Phe-Xaal-Xaal-Trp-Pro-Xaa2-Xaal-Xaal-Xaa2-Xaa2-Xaal-Xaal-Xaal-Xaal-Leu-Ala-Xaal-Ala-Gly-
 10 Phe-Tyr-Tyr-Xaal-Gly-Xaal-Xaal-Asp-Xaal-Val-Xaal-Cys-Phe-Xaal-Cys-Xaal-Xaal-Xaal-Xaal-Xaal-Xaal-Trp-Xaal-Xaal-Xaal-Asp-Xaal-Xaal-Xaal-Xaal-Xaal-His-Xaal-Xaal-Xaal-Xaal-Pro-Xaal-Cys-Xaal-Phe-Val, where Xaal may be any amino acid and Xaa2 may be any amino acid or may be
 15 absent (SEQ ID NO:2).

In various preferred embodiments the polypeptide has at least two or, more preferably at least three BIR domains, the RZF domain has one of the IAP sequences shown in Fig. 6, and the BIR domains are comprised of BIR
 20 domains shown in Fig. 5. In other preferred embodiments the BIR domains are at the amino terminal end of the protein relative to the RZF domain, which is at or near the carboxyl terminus of the polypeptide.

In another aspect, the invention features an IAP
 25 gene isolated according to the method involving: (a) providing a sample of DNA; (b) providing a pair of oligonucleotides having sequence homology to a conserved region of an IAP disease-resistance gene; (c) combining the pair of oligonucleotides with the cell DNA sample
 30 under conditions suitable for polymerase chain reaction-mediated DNA amplification; and (d) isolating the amplified IAP gene or fragment thereof.

In preferred embodiments, the amplification is carried out using a reverse-transcription polymerase
 35 chain reaction, for example, the RACE method. In another

- 7 -

aspect, the invention features an IAP gene isolated according to the method involving: (a) providing a preparation of DNA; (b) providing a detectably labelled DNA sequence having homology to a conserved region of an IAP gene; (c) contacting the preparation of DNA with the detectably-labelled DNA sequence under hybridization conditions providing detection of genes having 50% or greater nucleotide sequence identity; and (d) identifying an IAP gene by its association with the detectable label.

10 In another aspect, the invention features an IAP gene isolated according to the method involving: (a) providing a cell sample; (b) introducing by transformation into the cell sample a candidate IAP gene; (c) expressing the candidate IAP gene within the cell sample; and (d) determining whether the cell sample exhibits an altered apoptotic response, whereby a response identifies an IAP gene.

In another aspect, the invention features a method of identifying an IAP gene in a cell, involving: (a) providing a preparation of cellular DNA (for example, from the human genome or a cDNA library (such as a cDNA library isolated from a cell type which undergoes apoptosis); (b) providing a detectably-labelled DNA sequence (for example, prepared by the methods of the invention) having homology to a conserved region of an IAP gene; (c) contacting the preparation of cellular DNA with the detectably-labelled DNA sequence under hybridization conditions providing detection of genes having 50% nucleotide or greater sequence identity; and (d) identifying an IAP gene by its association with the detectable label.

In another aspect, the invention features a method of isolating an IAP gene from a recombinant library, involving: (a) providing a recombinant library; (b) contacting the library with a detectably-labelled

- 8 -

gene fragment produced according to the PCR method of the invention under hybridization conditions providing detection of genes having 50% or greater nucleotide sequence identity; and (c) isolating an IAP gene by its association with the detectable label. In another aspect, the invention features a method of identifying an IAP gene involving: (a) providing a cell tissue sample; (b) introducing by transformation into the cell sample a candidate IAP gene; (c) expressing the candidate IAP gene within the cell sample; and (d) determining whether the cell sample exhibits inhibition of apoptosis, whereby a change in (i.e. modulation of) apoptosis identifies an IAP gene. Preferably, the cell sample is a cell type that may be assayed for apoptosis (e.g., T cells, B cells, neuronal cells, baculovirus-infected insect cells, glial cells, embryonic stem cells, and fibroblasts). The candidate IAP gene is obtained, for example, from a cDNA expression library, and the response assayed is the inhibition of apoptosis.

In another aspect, the invention features a method of inhibiting apoptosis in a mammal wherein the method includes: (a) providing DNA encoding at least one IAP polypeptide to a cell that is susceptible to apoptosis; wherein the DNA is integrated into the genome of the cell and is positioned for expression in the cell; and the IAP gene is under the control of regulatory sequences suitable for controlled expression of the gene(s); wherein the IAP transgene is expressed at a level sufficient to inhibit apoptosis relative to a cell lacking the IAP transgene. The DNA integrated into the genome may encode all or part of an IAP polypeptide. It may, for example, encode a ring zinc finger and one or more BIR domains. In contrast, it may encode either the ring zinc finger alone, or one or more BIR domains alone. Skilled artisans will appreciate that IAP polypeptides

- 9 -

may also be administered directly to inhibit undesirable apoptosis.

In a related aspect, the invention features a method of inhibiting apoptosis by producing a cell that
5 has integrated, into its genome, a transgene that includes the IAP gene, or a fragment thereof. The IAP gene may be placed under the control of a promoter providing constitutive expression of the IAP gene. Alternatively, the IAP transgene may be placed under the
10 control of a promoter that allows expression of the gene to be regulated by environmental stimuli. For example, the IAP gene may be expressed using a tissue-specific or cell type-specific promoter, or by a promoter that is activated by the introduction of an external signal or
15 agent, such as a chemical signal or agent. In preferred embodiments the cell is a lymphocyte, a neuronal cell, a glial cell, or a fibroblast. In other embodiments, the cell in an HIV-infected human, or in a mammal suffering from a neurodegenerative disease, an ischemic injury, a
20 toxin-induced liver disease, or a myelodysplastic syndrome.

In a related aspect, the invention provides a method of inhibiting apoptosis in a mammal by providing an apoptosis-inhibiting amount of IAP polypeptide. The
25 IAP polypeptide may be a full-length polypeptide, or it may be one of the fragments described herein.

In another aspect, the invention features a purified antibody that binds specifically to an IAP family protein. Such an antibody may be used in any
30 standard immunodetection method for the identification of an IAP polypeptide. Preferably, the antibody binds specifically to XIAP, HIAP-1, or HIAP-2. In various embodiments, the antibody may react with other IAP polypeptides or may be specific for one or a few IAP
35 polypeptides. The antibody may be a monoclonal or a

- 10 -

polyclonal antibody. Preferably, the antibody reacts specifically with only one of the IAP polypeptides, for example, reacts with murine and human xiap, but not with hiap-1 or hiap-2 from other mammalian species.

5 The antibodies of the invention may be prepared by a variety of methods. For example, the IAP polypeptide, or antigenic fragments thereof, can be administered to an animal in order to induce the production of polyclonal antibodies. Alternatively,
10 antibodies used as described herein may be monoclonal antibodies, which are prepared using hybridoma technology (see, e.g., Kohler et al., Nature 256:495, 1975; Kohler et al., Eur. J. Immunol. 6:511, 1976; Kohler et al., Eur. J. Immunol. 6:292, 1976; Hammerling et al., In Monoclonal
15 Antibodies and T Cell Hybridomas, Elsevier, NY, 1981). The invention features antibodies that specifically bind human or murine IAP polypeptides, or fragments thereof. In particular the invention features "neutralizing" antibodies. By "neutralizing" antibodies is meant
20 antibodies that interfere with any of the biological activities of IAP polypeptides, particularly the ability of IAPs to inhibit apoptosis. The neutralizing antibody may reduce the ability of IAP polypeptides to inhibit polypeptides by, preferably 50%, more preferably by 70,
25 and most preferably by 90% or more. Any standard assay of apoptosis, including those described herein, may be used to assess neutralizing antibodies.

 In addition to intact monoclonal and polyclonal anti-IAP antibodies, the invention features various
30 genetically engineered antibodies, humanized antibodies, and antibody fragments, including F(ab')₂, Fab', Fab, Fv and sFv fragments. Antibodies can be humanized by methods known in the art, e.g., monoclonal antibodies with a desired binding specificity can be commercially
35 humanized (Scotgene, Scotland; Oxford Molecular, Palo

- 11 -

Alto, CA). Fully human antibodies, such as those expressed in transgenic animals, are also features of the invention (Green et al., Nature Genetics 7:13-21, 1994).

Ladner (U.S. Patent 4,946,778 and 4,704,692)

- 5 describes methods for preparing single polypeptide chain antibodies. Ward et al. (Nature 341:544-546, 1989) describe the preparation of heavy chain variable domains, which they term "single domain antibodies," which have high antigen-binding affinities. McCafferty et al. (Nature 348:552-554, 1990) show that complete antibody V domains can be displayed on the surface of fd bacteriophage, that the phage bind specifically to antigen, and that rare phage (one in a million) can be isolated after affinity chromatography. Boss et al. (U.S. Patent 4,816,397) describe various methods for producing immunoglobulines, and immunologically functional fragments thereof, which include at least the variable domains of the heavy and light chain in a single host cell. Cabilly et al. (U.S. Patent 4,816,567)
- 15 describe methods for preparing chimeric antibodies.

- In another aspect, the invention features a method of identifying a compound that modulates apoptosis. The method includes providing a cell expressing an IAP polypeptide, contacting the cell with a candidate compound, and monitoring the expression of an IAP gene. An alteration in the level of expression of the IAP gene indicates the presence of a compound which modulates apoptosis. The compound may be an inhibitor or an enhancer of apoptosis. In various preferred
- 25 embodiments, the cell is a fibroblast, a neuronal cell, a glial cell, a lymphocyte (T cell or B cell), or an insect cell; the polypeptide expression being monitored is XIAP, HIAP-1, HIAP-2, M-XIAP, M-HIAP-1, or M-HIAP-2 (i.e., human or murine).
- 30

- 12 -

In a related aspect, the invention features methods of detecting compounds that modulate apoptosis using the interaction trap technology and IAP polypeptides, or fragments thereof, as a component of the
5 bait. In preferred embodiments, the compound being tested as a modulator of apoptosis is also a polypeptide.

In another aspect, the invention features a method for diagnosing a cell proliferation disease, or an increased likelihood of such a disease, using an IAP
10 nucleic acid probe or antibody. Preferably, the disease is a cancer. Most preferably, the disease is selected from the group consisting of promyelocytic leukemia, a HeLa-type carcinoma, chronic myelogenous leukemia (preferably using xiap or hiap-2 related probes),
15 lymphoblastic leukemia (preferably using a xiap related probe), Burkitt's lymphoma (preferably using an hiap-1 related probe), colorectal adenocarcinoma, lung carcinoma, and melanoma (preferably using a xiap probe). Preferably, a diagnosis is indicated by a 2-fold increase
20 in expression or activity, more preferably, at least a 10-fold increase in expression or activity.

Skilled artisans will recognize that a mammalian IAP, or a fragment thereof (as described herein), may serve as an active ingredient in a therapeutic
25 composition. This composition, depending on the IAP or fragment included, may be used to modulate apoptosis and thereby treat any condition that is caused by a disturbance in apoptosis.

In addition, apoptosis may be induced in a cell
30 by administering to the cell a negative regulator of the IAP-dependent anti-apoptotic pathway. The negative regulator may be, but is not limited to, an IAP polypeptide that includes a ring zinc finger, and an IAP polypeptide that includes a ring zinc finger and lacks at
35 least one BIR domain. Alternatively, apoptosis may be

- 13 -

induced in the cell by administering a gene encoding an IAP polypeptide, such as these two polypeptides. In yet another method, the negative regulator may be a purified antibody, or a fragment thereof, that binds specifically to an IAP polypeptide. For example, the antibody may bind to an approximately 26 kDa cleavage product of an IAP polypeptide that includes at least one BIR domain but lacks a ring zinc finger domain. The negative regulator may also be an IAP antisense mRNA molecule.

As summarized above, an IAP nucleic acid, or an IAP polypeptide may be used to modulate apoptosis. Furthermore, an IAP nucleic acid, or an IAP polypeptide, may be used in the manufacture of a medicament for the modulation of apoptosis.

By "IAP gene" is meant a gene encoding a polypeptide having at least one BIR domain and a ring zinc finger domain which is capable of modulating (inhibiting or enhancing) apoptosis in a cell or tissue when provided by other intracellular or extracellular delivery methods. In preferred embodiments the IAP gene is a gene having about 50% or greater nucleotide sequence identity to at least one of the IAP amino acid encoding sequences of Figs. 1-4 or portions thereof. Preferably, the region of sequence over which identity is measured is a region encoding at least one BIR domain and a ring zinc finger domain. Mammalian IAP genes include nucleotide sequences isolated from any mammalian source. Preferably, the mammal is a human.

The term "IAP gene" is meant to encompass any member of the family of apoptosis inhibitory genes, which are characterized by their ability to modulate apoptosis. An IAP gene may encode a polypeptide that has at least 20%, preferably at least 30%, and most preferably at least 50% amino acid sequence identity with at least one of the conserved regions of one of the IAP members

- 14 -

described her in (i.e., either the BIR or ring zinc finger domains from the human or murine xiap, hiap-1 and hiap-2). Representative members of the IAP gene family include, without limitation, the human and murine xiap, 5 hiap-1, and hiap-2 genes.

By "IAP protein" or "IAP polypeptide" is meant a polypeptide, or fragment thereof, encoded by an IAP gene.

By "BIR domain" is meant a domain having the amino acid sequence of the consensus sequence: Xaal-Xaal-
10 Xaal-Arg-Leu-Xaal-Thr-Phe-Xaal-Xaal-Trp-Pro-Xaa2-Xaal-
Xaal-Xaa2-Xaa2-Xaal-Xaal-Xaal-Xaal-Leu-Ala-Xaal-Ala-Gly-
Phe-Tyr-Tyr-Xaal-Gly-Xaal-Xaal-Asp-Xaal-Val-Xaal-Cys-Phe-
Xaal-Cys-Xaal-Xaal- Xaal-Xaal-Xaal-Xaal-Trp-Xaal-Xaal-
Xaal-Asp-Xaal-Xaal-Xaal- Xaal-Xaal-His-Xaal-Xaal-Xaal-
15 Xaal-Pro-Xaal-Cys-Xaal-Phe-Val, wherein Xaal is any amino acid and Xaa2 is any amino acid or is absent (SEQ ID NO:2). Preferably, the sequence is substantially identical to one of the BIR domain sequences provided for xiap, hiap-1, hiap-2 herein.

20 By "ring zinc finger" or "RZF" is meant a domain having the amino acid sequence of the consensus sequence: Glu-Xaal-Xaal-Xaal-Xaal-Xaal-Xaal-Xaa2-Xaal-Xaal-Xaal-Cys- Lys-Xaa3-Cys-Met-Xaal-Xaal-Xaal-Xaal-Xaal-Xaa3-Xaal-Phe-Xaal-Pro-Cys-Gly-His-Xaal-Xaal-Xaal-Cys-Xaal-Xaal-
25 Cys-Ala- Xaal-Xaal-Xaal-Xaal-Xaal-Cys-Pro-Xaal-Cys, wherein Xaal is any amino acid, Xaa2 is Glu or Asp, and Xaa3 is Val or Ile (SEQ ID NO:1).

Preferably, the sequence is substantially identical to the RZF domains provided herein for the
30 human or murine xiap, hiap-1, or hiap-2.

By "modulating apoptosis" or "altering apoptosis" is meant increasing or decreasing the number of cells that would otherwise undergo apoptosis in a given cell population. Preferably, the cell population
35 is selected from a group including T cells, neuronal

- 15 -

cells, fibroblasts, or any other cell line known to undergo apoptosis in a laboratory setting (e.g., the baculovirus infected insect cells). It will be appreciated that the degree of modulation provided by an IAP or modulating compound in a given assay will vary, but that one skilled in the art can determine the statistically significant change in the level of apoptosis which identifies an IAP or a compound which modulates an IAP.

By "inhibiting apoptosis" is meant any decrease in the number of cells which undergo apoptosis relative to an untreated control. Preferably, the decrease is at least 25%, more preferably the decrease is 50%, and most preferably the decrease is at least one-fold.

By "polypeptide" is meant any chain of more than two amino acids, regardless of post-translational modification such as glycosylation or phosphorylation.

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 50%, preferably 85%, more preferably 90%, and most preferably 95% homology to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

Sequence identity is typically measured using sequence analysis software with the default parameters specified therein (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). This software program matches

- 16 -

similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups:

- 5 glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "substantially pure polypeptide" is meant a polypeptide that has been separated from the components
10 that naturally accompany it. Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated.

Preferably, the polypeptide is an IAP polypeptide that is
15 at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, pure. A substantially pure IAP polypeptide may be obtained, for example, by extraction from a natural source (e.g. a fibroblast, neuronal cell, or lymphocyte) by expression
20 of a recombinant nucleic acid encoding an IAP polypeptide, or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

25 A protein is substantially free of naturally associated components when it is separated from those contaminants which accompany it in its natural state. Thus, a protein which is chemically synthesized or produced in acellular system different from the cell from
30 which it naturally originates will be substantially free from its naturally associated components. Accordingly, substantially pure polypeptides include those derived from eukaryotic organisms but synthesized in *E. coli* or other prokaryotes. By "substantially pure DNA" is meant
35 DNA that is free of the genes which, in the naturally-

- 17 -

occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously
5 replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a
10 recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule
15 encoding (as used herein) an IAP polypeptide.

By "transgene" is meant any piece of DNA which is inserted by artifice into a cell, and becomes part of the genome of the organism which develops from that cell. Such a transgene may include a gene which is partly or
20 entirely heterologous (i.e., foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism.

By "transgenic" is meant any cell which includes a DNA sequence which is inserted by artifice into a cell
25 and becomes part of the genome of the organism which develops from that cell. As used herein, the transgenic organisms are generally transgenic mammalian (e.g., rodents such as rats or mice) and the DNA (transgene) is inserted by artifice into the nuclear genome.

30 By "transformation" is meant any method for introducing foreign molecules into a cell. Lipofection, calcium phosphate precipitation, retroviral delivery, electroporation, and biolistic transformation are just a few of the teachings which may be used. For example,
35 biolistic transformation is a method for introducing

- 18 -

foreign molecules into a cell using velocity driven microprojectil s such as tungsten or gold particles. Such velocity-driven methods originate from pressure bursts which include, but are not limited to, helium-
5 driven, air-driven, and gunpowder-driven techniques. Biolistic transformation may be applied to the transformation or transfection of a wide variety of cell types and intact tissues including, without limitation, intracellular organelles (e.g., and mitochondria and
10 chloroplasts), bacteria, yeast, fungi, algae, animal tissue, and cultured cells.

By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the
15 sequence (i.e., facilitates the production of, e.g., an IAP polypeptide, a recombinant protein or a RNA molecule).

By "reporter gene" is meant a gene whose expression may be assayed; such genes include, without
20 limitation, glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), and β -galactosidase.

By "promoter" is meant minimal sequence sufficient to direct transcription. Also included in the
25 invention are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell type-specific, tissue-specific or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the native
30 gene.

By "operably linked" is meant that a gene and one or more regulatory sequences are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins are
35 bound to the regulatory sequences).

- 19 -

By "conserved region" is meant any stretch of six or more contiguous amino acids exhibiting at least 30%, preferably 50%, and most preferably 70% amino acid sequence identity between two or more of the IAP family members, (e.g., between human HIAP-1, HIAP-2, and XIAP). Examples of preferred conserved regions are shown (as boxed or designated sequences) in Figures 5-7 and Tables 1 and 2, and include, without limitation, BIR domains and ring zinc finger domains.

By "detectably-labelled" is meant any means for marking and identifying the presence of a molecule, e.g., an oligonucleotide probe or primer, a gene or fragment thereof, or a cDNA molecule. Methods for detectably-labelling a molecule are well known in the art and include, without limitation, radioactive labelling (e.g., with an isotope such as ^{32}P or ^{35}S) and nonradioactive labelling (e.g., chemiluminescent labelling, e.g., fluorescein labelling).

By "antisense," as used herein in reference to nucleic acids, is meant a nucleic acid sequence, regardless of length, that is complementary to the coding strand of a gene.

By "purified antibody" is meant antibody which is at least 60%, by weight, free from proteins and naturally occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight, antibody, e.g., an IAP specific antibody. A purified antibody may be obtained, for example, by affinity chromatography using recombinantly-produced protein or conserved motif peptides and standard techniques.

By "specifically binds" is meant an antibody that recognizes and binds a protein but that does not substantially recognize and bind other molecules in a

- 20 -

sample, e.g., a biological sample, that naturally includes protein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Drawings

Fig. 1 is the human xiap cDNA sequence (SEQ ID NO:3) and the XIAP polypeptide sequence (SEQ ID NO:4).

10 Fig. 2 is the human hiap-1 cDNA sequence (SEQ ID NO:5) and the HIAP-1 polypeptide sequence (SEQ ID NO:6).

Fig. 3 is the human hiap-2 cDNA sequence (SEQ ID NO:7) and the HIAP-2 polypeptide sequence (SEQ ID NO:8). The sequence absent in the hiap-2-Δ variant is boxed.

Fig. 4 is the murine xiap cDNA sequence (SEQ ID NO:9) and encoded murine XIAP polypeptide sequence (SEQ ID NO:10).

20 Fig. 5 is the murine hiap-1 cDNA sequence (SEQ ID NO:39) and the encoded murine HIAP-1 polypeptide sequence (SEQ ID NO:40).

Fig. 6 is the murine hiap-2 cDNA sequence (SEQ ID NO:41) and the encoded murine HIAP-2 polypeptide (SEQ ID NO:42).

25 Fig. 7 is a representation of the alignment of the BIR domains of IAP proteins (SEQ ID NOs 11 and 14-31).

Fig. 8 is a representation of the alignment of human IAP polypeptides with diap, cp-iap, and the IAP consensus sequence (SEQ ID NOs:4, 6, 8, 10, 12, and 13).

30 Fig. 9 is a representation of the alignment of the ring zinc finger domains of IAP proteins (SEQ ID NOs:32-38).

- 21 -

Fig. 10 is a photograph of a Northern blot illustrating human hiap-1 and hiap-2 mRNA expression in human tissues.

Fig. 11 is a photograph of a Northern blot
5 illustrating human hiap-2 mRNA expression in human tissues.

Fig. 12 is a photograph of a Northern blot illustrating human xiap mRNA expression in human tissues.

Fig. 13A and 13B are photographs of agarose gels
10 illustrating apoptotic DNA ladders and RT-PCR products using hiap-1 and hiap-2 specific probes in HIV-infected T cells.

Fig. 14A - 14D are graphs depicting suppression of apoptosis by XIAP, HIAP-1, HIAP-2, bcl-2, smn, and 6-
15 myc.

Fig. 15A - 15B are bar graphs depicting the percentage of viable CHO cells following transient transfection with the cDNA constructs shown and subsequent serum withdrawal.

Fig. 16A - 16B are bar graphs depicting the
20 percentage of viable CHO cells following transient transfection with the cDNA constructs shown and subsequent exposure to menadione (Fig. 16A = 10 μ M menadione; Fig. 16B = 20 μ M menadione).

Fig. 17 is a photograph of an agarose gel
25 containing cDNA fragments that were amplified, with hiap-1-specific primers, from RNA obtained from Raji, Ramos, EB-3, and Jiyoye cells, and from normal placenta.

Fig. 18 is a photograph of a Western blot
30 containing protein extracted from Jurkat and astrocytoma cells stained with an anti-XIAP antibody. The position and size of a series of marker proteins is indicated.

Fig. 19 is a photograph of a Western blot containing protein extracted from Jurkat cells following
35 treatment as described in Example XII. The blot was

- 22 -

stained with a rabbit polyclonal anti-XIAP antibody. Lane 1, negative control; lane 2, anti-Fas antibody; lane 3, anti-Fas antibody and cycloheximide; lane 4, TNF- α ; lane 5, TNF- α and cycloheximide.

5 Fig. 20 is a photograph of a Western blot containing protein extracted from HeLa cells following exposure to anti-Fas antibodies. The blot was stained with a rabbit polyclonal anti-XIAP antibody. Lane 1, negative control; lane 2, cycloheximide; lane 3, anti-Fas
10 antibody; lane 4, anti-Fas antibody and cycloheximide; lane 5, TNF- α ; lane 6, TNF- α and cycloheximide.

Fig. 21A - 21B are photographs of Western blots stained with rabbit polyclonal anti-XIAP antibody. Protein was extracted from HeLa cells (Fig. 21A) and
15 Jurkat cells (Fig. 21B) immediately, 1, 2, 3, 5, 10, and 22 hours after exposure to anti-Fas antibody.

Fig. 22A and 22B are photographs of Western blots stained with an anti-CPP32 antibody (Fig. 22A) or a rabbit polyclonal anti-XIAP antibody (Fig. 22B). Protein
20 was extracted from Jurkat cells immediately, 3 hours, or 7 hours after exposure to an anti-Fas antibody. In addition to total protein, cytoplasmic and nuclear extracts are shown.

Fig. 23 is a photograph of a polyacrylamide gel
25 following electrophoresis of the products of an *in vitro* XIAP cleavage assay.

Detailed Description

I. IAP Genes and Polypeptides

A new class of mammalian proteins that modulate
30 apoptosis (IAPS) and the genes that encode these proteins have been discovered. The IAP proteins are characterized by the presence of a ring zinc finger domain (RZF; Fig. 9) and at least one BIR domain, as defined by the boxed consensus sequences shown in Figs. 7 and 8, and by the

- 23 -

sequence domains listed in Tables 1 and 2. As examples of novel IAP genes and proteins, the cDNA sequences and amino acid sequences for human IAPs (HIAP-1, HIAP-2, and XIAP) and a new murine inhibitor of apoptosis, XIAP, are provided. Additional members of the mammalian IAP family (including homologs from other species and mutant sequences) may be isolated using standard cloning techniques and the conserved amino acid sequences, primers, and probes provided herein and known in the art. Furthermore, IAPs include those proteins lacking the ring zinc finger, as further described below.

TABLE 1
NUCLEOTIDE POSITION OF CONSERVED DOMAINS*

	BIR-1	BIR-2	BIR-3	Ring Zinc Finger
h-xiap	109 - 312	520 - 723	826 - 1023	1348 - 1485
m-xiap	202 - 405	613 - 816	916 - 1113	1438 - 1575
h-hiap-1	273 - 476	693 - 893	951 - 1154	1824 - 1961
m-hiap-1	251 - 453	670 - 870	928 - 1131	1795 - 1932
h-hiap-2	373 - 576	787 - 987	1042 - 1245	1915 - 2052
m-hiap-2	215 - 418	608 - 808	863 - 1066	1763 - 1876

*Positions indicated correspond to those shown in Figs. 1-4.

- 24 -

TABLE 2

AMINO ACID POSITION OF CONSERVED DOMAINS*

	BIR-1	BIR-2	BIR-3	Ring Zinc Finger
h-XAIP	26 - 93	163 - 230	265 - 330	439 - 484
m-XIAP	26 - 93	163 - 230	264 - 329	438 - 483
h-HIAP1	29 - 96	169 - 235	255 - 322	546 - 591
m-HIAP1	29 - 96	169 - 235	255 - 322	544 - 589
h-HIAP2	46 - 113	184 - 250	269 - 336	560 - 605
m-HIAP2	25 - 92	156 - 222	241 - 308	541 - 578

*Positions indicated correspond to those shown in Figs.
1-4.

Recognition of the mammalian IAP family has provided an emergent pattern of protein structure. Recognition of this pattern allows proteins having a known, homologous sequence but unknown function to be classified as putative inhibitors of apoptosis. A drosophila gene, now termed diap, was classified in this way (for sequence information see Genbank Accession Number M96581 and Fig. 6). The conservation of these proteins across species indicates that the apoptosis signalling pathway has been conserved throughout evolution.

The IAP proteins may be used to inhibit the apoptosis that occurs as part of numerous disease processes or disorders. For example, IAP polypeptides or nucleic acid encoding IAP polypeptides may be administered for the treatment or prevention of apoptosis that occurs as a part of AIDS, neurodegenerative diseases, ischemic injury, toxin-induced liver disease and myelodysplastic syndromes. Nucleic acid encoding the IAP polypeptide may also be provided to inhibit apoptosis.

- 25 -

II. Cloning of IAP Genes

A. xiap

The search for human genes involved in apoptosis resulted in the identification of an X-linked sequence tag site (STS) in the GenBank database, which demonstrated strong homology with the conserved RZF domain of CpIAP and OpIAP, the two baculovirus genes known to inhibit apoptosis (Clem et al., Mol. Cell Biol. 14:5212-5222, 1994; Birnbaum et al., J. Virol. 68:2521-8, 1994). Screening a human fetal brain ZapII cDNA library (Stratagene, La Jolla, CA) with this STS resulted in the identification and cloning of xiap (for X-linked Inhibitor of Apoptosis Protein gene). The human gene has a 1.5 kb coding sequence that includes three BIR domains (Crook et al., J. Virol. 67:2168-74, 1993; Clem et al., Science 254:1388-90, 1991; Birnbaum et al., J. Virol., 68:2521-8, 1994) and a zinc finger. Northern blot analysis with xiap revealed message greater than 7 kb, which is expressed in various tissues, particularly liver and kidney (Fig. 12). The large size of the transcript reflects large 5' and 3' untranslated regions.

B. Human hiap-1 and hiap-2

The hiap-1 and hiap-2 genes were cloned by screening a human liver library (Stratagene Inc., LaJolla, CA) with a probe including the entire xiap coding region at low stringency (the final wash was performed at 40°C with 2X SSC, 10% SDS; Figs. 2 and 3). The hiap-1 and hiap-2 genes were also detected independently using a probe derived from an expressed sequence tag (EST; GenBank Accession No. T96284), which includes a portion of a BIR domain. The EST sequence was originally isolated by the polymerase chain reaction; a cDNA library was used as a template and amplified with EST-specific primers. The DNA amplimer probe was then used to screen the human liver cDNA library for

- 26 -

full-length hiap coding sequences. A third DNA was subsequently detected that includes the hiap-2 sequence but that appears to lack one exon, presumably due to alternative mRNA splicing (see boxed region in Fig. 3).

- 5 The expression of hiap-1 and hiap-2 in human tissues as assayed by Northern blot analysis is shown in Figures 8 and 9.

C. m-xiap

Fourteen cDNA and two genomic clones were
10 identified by screening a mouse embryo λ gt11 cDNA library (Clontech, Palo Alto, CA) and a mouse FIX II genomic library with a xiap cDNA probe, respectively. A cDNA contig spanning 8.0 kb was constructed using 12 overlapping mouse clones. Sequence analysis revealed a
15 coding sequence of approximately 1.5 kb. The mouse gene, m-xiap, encodes a polypeptide with striking homology to human XIAP at and around the initiation methionine, the stop codon, the three BIR domains, and the RZF domain. As with the human gene, the mouse homologue contains
20 large 5' and 3' UTRs, which could produce a transcript as large as 7-8 kb.

Analysis of the sequence and restriction map of m-xiap further delineate the structure and genomic organization of m-xiap. Southern blot analysis and
25 inverse PCR techniques (Grodén et al., Cell 66:589-600, 1991) can be employed to map exons and define exon-intron boundaries.

Antisera can be raised against a m-xiap fusion protein that was obtained from, for example, *E. coli*
30 using a bacterial expression system. The resulting antisera can be used along with Northern blot analysis to analyze the spatial and temporal expression of m-xiap in the mouse.

- 27 -

D. m-hiap-1 and m-hiap-2

The murine homologs of hiap-1 and hiap-2 were cloned and sequenced in the same general manner as m-xiap using the human hiap-1 and hiap-2 sequences as probes.

- 5 Cloning of m-hiap-1 and m-hiap-2 further demonstrate that homologs from different species may be isolated using the techniques provided herein and those generally known to artisans skilled in molecular biology.

III. Identification of Additional IAP Genes

- 10 Standard techniques, such as the polymerase chain reaction (PCR) and DNA hybridization, may be used to clone additional human IAP genes and their homologues in other species. Southern blots of human genomic DNA hybridized at low stringency with probes specific for
15 xiap, hiap-1 and hiap-2 reveal bands that correspond to other known human IAP sequences as well as additional bands that do not correspond to known IAP sequences. Thus, additional IAP sequences may be readily identified using low stringency hybridization. Examples of murine
20 and human xiap, hiap-1, and hiap-2 specific primers, which may be used to clone additional genes by RT-PCR, are shown in Table 5.

IV. Characterization of IAP Activity and Intracellular Localization Studies

- 25 The ability of putative IAPs to modulate apoptosis can be defined in in vitro systems in which alterations of apoptosis can be detected. Mammalian expression constructs carrying IAP cDNAs, which are either full-length or truncated, can be introduced into
30 cell lines such as CHO, NIH 3T3, HL60, Rat-1, or Jurkat cells. In addition, SF21 insect cells may be used, in which case the IAP gene is preferentially expressed using an insect heat shock promotor. Following transfection, apoptosis can be induced by standard methods, which
35 include serum withdrawal, or application of staurosporine, menadione (which induces apoptosis via

- 28 -

free radical formation), or anti-Fas antibodies. As a control, cells are cultured under the same conditions as those induced to undergo apoptosis, but either not transfected, or transfected with a vector that lacks an IAP insert. The ability of each IAP construct to inhibit apoptosis upon expression can be quantified by calculating the survival index of the cells, i.e., the ratio of surviving transfected cells to surviving control cells. These experiments can confirm the presence of apoptosis inhibiting activity and, as discussed below, can also be used to determine the functional region(s) of an IAP. These assays may also be performed in combination with the application of additional compounds in order to identify compounds that modulate apoptosis via IAP expression.

A. Cell Survival following Transfection
with Full-length IAP Constructs
and Induction of Apoptosis

Specific examples of the results obtained by performing various apoptosis suppression assays are shown in Figs. 14A to 14D. For example, CHO cell survival following transfection with one of six constructs and subsequent serum withdrawal is shown in Fig. 14A. The cells were transfected using Lipofectace™ with 2 µg of one of the following recombinant plasmids: pCDNA36myc-xiap (xiap), pCDNA3-6myc-hiap-1 (hiap-1), pCDNA3-6myc-hiap-2 (hiap-2), pCDNA3-bcl-2 (bcl-2), pCDNA3-HA-smn (smn), and pCDNA3-6myc (6-myc). Oligonucleotide primers were synthesized to allow PCR amplification and cloning of the xiap, hiap-1, and hiap-2 ORFs in pCDNA3 (Invitrogen). Each construct was modified to incorporate a synthetic myc tag encoding six repeats of the peptide sequence MEQKLISEEDL [(SEQ ID NO:___)], thus allowing detection of myc-IAP fusion proteins via monoclonal anti-myc antiserum (Egan et al., Nature 363:45-51, 1993). Triplicate samples of cell lines in 24-well dishes were

- 29 -

washed 5 times with serum free media and maintained in serum free conditions during the course of the experiment. Cells that excluded trypan blue, and that were therefore viable, were counted with a hemocytometer immediately, 24 hours, 48 hours, and 72 hours, after serum withdrawal. Survival was calculated as a percentage of the initial number of viable cells. In this experiment and those presented in Figs. 14B and 14D, the percentage of viable cells shown represents the average of three separate experiments performed in triplicate, +/- average deviation.

The survival of CHO cells following transfection (with each one of the six constructs described above) and exposure to menadione is shown in Fig. 14B. The cells were plated in 24-well dishes, allowed to grow overnight, and then exposed to 20 μ M menadione for 1.5 hours (Sigma Chemical Co., St. Louis, MO). Triplicate samples were harvested at the time of exposure to menadione and 24 hours afterward, and survival was assessed by trypan blue exclusion.

The survival of Rat-1 cells following transfection (with each one of the six constructs described above) and exposure to staurosporine is shown in Fig. 14C. Rat-1 cells were transfected and then selected in medium containing 800 μ g/ml G418 for two weeks. The cell line was assessed for resistance to staurosporine-induced apoptosis (1 μ M) for 5 hours. Viable cells were counted 24 hours after exposure to staurosporine by trypan blue exclusion. The percentage of viable cells shown represents the average of two experiments, \pm average deviation.

The Rat-1 cell line was also used to test the resistance of these cells to menadione (Fig. 14D) following transfection with each of the six constructs described above. The cells were exposed to 10 μ M

- 30 -

menadione for 1.5 hours, and the number of viable cells was counted 18 hours later.

B. Comparison of Cell Survival
Following Transfection with
Full-length vs. Partial IAP Constructs

5 In order to investigate the mechanism whereby human IAPs, including XIAP, HIAP-1, and HIAP-2, afford protection against cell death, expression vectors were constructed that contained either: (1) full-length IAP
10 cDNA (as described above), (2) a portion of an IAP gene that encodes the BIR domains, but not the RZF, or (3) a portion of an IAP gene that encodes the RZF, but not the BIR domains. Human and murine xiap or m-xiap cDNAs were tested by transient or stable expression in HeLa, Jurkat,
15 and CHO cell lines. Following transfection, apoptosis was induced by serum withdrawal, application of menadione, or application of an anti-Fas antibody. Cell death was then assessed, as described above, by trypan blue exclusion. As a control for transfection
20 efficiency, the cells were co-transfected with a β -gal expression construct. Typically, approximately 20% of the cells were successfully transfected.

When CHO cells were transiently transfected, constructs containing full-length xiap or m-xiap cDNAs
25 conferred modest protection against cell death (Fig. 15A). In contrast, the survival of CHO cells transfected with constructs encoding only the BIR domains (i.e., lacking the RZF domain; see Fig. 15A) was markedly enhanced 72 hours after serum deprivation. Furthermore,
30 a large percentage of cells expressing the BIR domains were still viable after 96 hours, at which time no viable cells remained in the control, i.e. non-transfected, cell cultures (see "CHO" in Fig. 15A), and less than 5% of the cells transfected with the vector only, i.e., lacking a
35 cDNA insert, remained viable (see "pcDNA3" in Fig. 15A). Deletion of any of the BIR domains results in the

- 31 -

complete loss of apoptotic suppression, which is reflected by a decrease in the percentage of surviving CHO cells to control levels within 72 hours of serum withdrawal (Fig. 15B; see "xiap Δ 1" (which encodes amino acids 89-497 of XIAP (SEQ ID NO.:4)), "xiap Δ 2" (which encodes amino acids 246-497 of XIAP (SEQ ID NO.:4)), and "xiap Δ 3" (which encodes amino acids 342-497 of XIAP (SEQ ID NO.:4)) at 72 hours).

Stable pools of transfected CHO cells, which were maintained for several months under G418 selection, were induced to undergo apoptosis by exposure to 10 μ M menadione for 2 hours. Among the CHO cells tested were those that were stably transfected with: (1) full-length m-xiap cDNA (miap), (2) full-length xiap cDNA (xiap), (3) full-length bcl-2 cDNA (Bcl-2), (4) cDNA encoding the three BIR domains (but not the RZF) of m-xiap (BIR), and (5) cDNA encoding the RZF (but not BIR domains) of m-xiap (RZF). Cells that were non-transfected (CHO) or transfected with the vector only (pcDNA3), served as controls for this experiment. Following exposure to 10 μ M menadione, the transfected cells were washed with phosphate buffered saline (PBS) and cultured for an additional 24 hours in menadione-free medium. Cell death was assessed, as described above, by trypan blue exclusion. Less than 10% of the non-transfected or vector-only transfected cells remained viable at the end of the 24 hour survival period. Cells expressing the RZF did not fare significantly better. However, expression of full-length m-xiap, xiap, or bcl-2, and expression of the BIR domains, enhanced cell survival (Fig. 16A). When the concentration of menadione was increased from 10 μ M to 20 μ M (with all other conditions of the experiment being the same as when 10 μ M menadione was applied), the percentage of viable CHO cells that expressed the BIR domain cDNA construct was higher than the percentage of

- 32 -

viable cells that expressed either full-length m-xiap or bcl-2 (Fig. 16B).

C. Analysis of the Subcellular Location
of Expressed RZF and BIR Domains

5 The assays of cell death described above indicate that the RZF may act as a negative regulator of the anti-apoptotic function of IAPs. One way in which the RZF, and possibly other IAP domains, may exert their regulatory influence is by altering the expression of
10 genes, whose products function in the apoptotic pathway.

 In order to determine whether the subcellular locations of expressed RZF and BIR domains are consistent with roles as nuclear regulatory factors, COS cells were transiently transfected with the following four
15 constructs, and the expressed polypeptide was localized by immunofluorescent microscopy: (1) pcDNA3-6myc-xiap, which encodes all 497 amino acids of SEQ ID NO:4, (2) pcDNA3-6myc-m-xiap, which encodes all 497 amino acids of mouse xiap (SEQ ID NO:10), (3) pcDNA3-6myc-mxiap-BIR,
20 which encodes amino acids 1 to 341 of m-xiap (SEQ ID NO:10), and (4) pcDNA3-6myc-mxiap-RZF, which encodes amino acids 342-497 of m-xiap (SEQ ID NO:10). The cells were grown on multi-well tissue culture slides for 12 hours, and then fixed and permeabilized with methanol.
25 The constructs used (here and in the cell death assays) were tagged with a human Myc epitope tag at the N-terminus. Therefore, a monoclonal anti-Myc antibody and a secondary goat anti-mouse antibody, which was conjugated to FITC, could be used to localize the
30 expressed products in transiently transfected COS cells. Full-length XIAP and MIAP were located in the cytoplasm, with accentuated expression in the peri-nuclear zone. The same pattern of localization was observed when the cells expressed a construct encoding the RZF domain (but
35 not the BIR domains). However, cells expressing the BIR domains (without the RZF) exhibited, primarily, nuclear

- 33 -

staining. The protein expressed by the BIR domain construct appeared to be in various stages of transfer to the nucleus.

These observations are consistent with the fact that, as described below, XIAP is cleaved within T cells that are treated with anti-Fas antibodies (which are potent inducers of apoptosis), and its N-terminal domain is translocated to the nucleus.

D. Examples of Additional Apoptosis Assays

Specific examples of apoptosis assays are also provided in the following references. Assays for apoptosis in lymphocytes are disclosed by: Li et al., "Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein", Science 268:429-431, 1995; Gibellini et al., "Tat-expressing Jurkat cells show an increased resistance to different apoptotic stimuli, including acute human immunodeficiency virus-type 1 (HIV-1) infection", Br. J. Haematol. 89:24-33, 1995; Martin et al., "HIV-1 infection of human CD4⁺ T cells in vitro. Differential induction of apoptosis in these cells." J. Immunol. 152:330-42, 1994; Terai et al., "Apoptosis as a mechanism of cell death in cultured T lymphoblasts acutely infected with HIV-1", J. Clin Invest. 87:1710-5, 1991; Dhein et al., "Autocrine T-cell suicide mediated by APO-1/(Fas/CD95)11, Nature 373:438-441, 1995; Katsikis et al., "Fas antigen stimulation induces marked apoptosis of T lymphocytes in human immunodeficiency virus-infected individuals", J. Exp. Med. 181:2029-2036, 1995; Westendorp et al., "Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120", Nature 375:497, 1995; DeRossi et al., Virology 198:234-44, 1994.

Assays for apoptosis in fibroblasts are disclosed by: Vossbeck et al., "Direct transforming activity of TGF-beta on rat fibroblasts", Int. J. Cancer 61:92-97, 1995; Goruppi et al., "Dissection of c-myc

- 34 -

domains involved in S phase induction of NIH3T3 fibroblasts", *Oncogene* 9:1537-44, 1994; Fernandez et al., "Differential sensitivity of normal and Ha-ras transformed C3H mouse embryo fibroblasts to tumor
5 necrosis factor: induction of bcl-2, c-myc, and manganese superoxide dismutase in resistant cells", *Oncogene* 9:2009-17, 1994; Harrington et al., "c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines", *EMBO J.*, 13:3286-3295, 1994; Itoh et al., "A
10 novel protein domain required for apoptosis. Mutational analysis of human Fas antigen", *J. Biol. Chem.* 268:10932-7, 1993.

Assays for apoptosis in neuronal cells are disclosed by: Melino et al., "Tissue transglutaminase
15 and apoptosis: sense and antisense transfection studies with human neuroblastoma cells", *Mol. Cell Biol.* 14:6584-6596, 1994; Rosenbaum et al., "Evidence for hypoxia-induced, programmed cell death of cultured neurons", *Ann. Neurol.* 36:864-870, 1994; Sato et al., "Neuronal
20 differentiation of PC12 cells as a result of prevention of cell death by bcl-2", *J. Neurobiol* 25:1227-1234, 1994; Ferrari et al., "N-acetylcysteine D- and L-stereoisomers prevents apoptotic death of neuronal cells", *J. Neurosci.* 15:2857-2866, 1995; Talley et al., "Tumor necrosis
25 factor alpha-induced apoptosis in human neuronal cells: protection by the antioxidant N-acetylcysteine and the genes bcl-2 and crma", *Mol. Cell Biol.* 15:2359-2366, 1995; Talley et al., "Tumor Necrosis Factor Alpha-Induced Apoptosis in Human Neuronal Cells: Protection by the
30 Antioxidant NAcetylcysteine and the Genes bcl-2 and crma", *Mol. Cell. Biol.* 15:2359-2366, 1995; Walkinshaw et al., "Induction of apoptosis in catecholaminergic PC12 cells by L-DOPA. Implications for the treatment of Parkinson's disease.", *J. Clin. Invest.* 95:2458-2464,
35 1995.

- 35 -

Assays for apoptosis in insect cells are disclosed by: Clem et al., "Prevention of apoptosis by a baculovirus gene during infection of insect cells", Science 254:1388-90, 1991; Crook et al., "An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif", J. Virol. 67:2168-74, 1993; Rabizadeh et al., "Expression of the baculovirus p35 gene inhibits mammalian neural cell death", J. Neurochem. 61:2318-21, 1993; Birnbaum et al., "An apoptosis inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs", J. Virol. 68:2521-8, 1994; Clem et al., "Control of programmed cell death by the baculovirus genes p35 and IAP", Mol. Cell. Biol. 14:5212-5222, 1994.

15 V. Construction of a Transgenic Animal
Characterization of IAP genes provides information that is necessary for an IAP knockout animal model to be developed by homologous recombination. Preferably, the model is a mammalian animal, most
20 preferably a mouse. Similarly, an animal model of IAP overproduction may be generated by integrating one or more IAP sequences into the genome, according to standard transgenic techniques.

A replacement-type targeting vector, which would
25 be used to create a knockout model, can be constructed using an isogenic genomic clone, for example, from a mouse strain such as 129/Sv (Stratagene Inc., LaJolla, CA). The targeting vector will be introduced into a suitably-derived line of embryonic stem (ES) cells by
30 electroporation to generate ES cell lines that carry a profoundly truncated form of an IAP. To generate chimeric founder mice, the targeted cell lines will be injected into a mouse blastula stage embryo. Heterozygous offspring will be interbred to homozygosity.
35 Knockout mice would provide the means, in vivo, to screen

- 36 -

for therapeutic compounds that modulate apoptosis via an IAP-dependent pathway.

VI. IAP Protein Expression

IAP genes may be expressed in both prokaryotic
5 and eukaryotic cell types. If an IAP modulates apoptosis by exacerbating it, it may be desirable to express that protein under control of an inducible promotor.

In general, IAPs according to the invention may
10 be produced by transforming a suitable host cell with all or part of an IAP-encoding cDNA fragment that has been placed into a suitable expression vector.

Those skilled in the art of molecular biology will understand that a wide variety of expression systems
15 may be used to produce the recombinant protein. The precise host cell used is not critical to the invention. The IAP protein may be produced in a prokaryotic host (e.g., *E. coli*) or in a eukaryotic host (e.g., *S. cerevisiae*, insect cells such as Sf21 cells, or mammalian
20 cells such as COS-1, NIH 3T3, or HeLa cells). These cells are publically available, for example, from the American Type Culture Collection, Rockville, MD; see also Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, 1994). The method of
25 transduction and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel et al. (*supra*), and expression vehicles may be chosen from those provided, e.g. in Cloning Vectors: A Laboratory
30 Manual (P.H. Pouwels et al., 1985, Supp. 1987).

A preferred expression system is the baculovirus system using, for example, the vector pBacPAK9, which is available from Clontech (Palo Alto, CA). If desired, this system may be used in conjunction with other protein
35 expression techniques, for example, the myc tag approach

- 37 -

described by Evan et al. (Mol. Cell Biol. 5:3610-3616, 1985).

Alternatively, an IAP may be produced by a stably-transfected mammalian cell line. A number of
5 vectors suitable for stable transfection of mammalian cells are available to the public, e.g., see Pouwels et al. (*supra*), as are methods for constructing such cell lines (see e.g., Ausubel et al. (*supra*). In one example, cDNA encoding an IAP is cloned into an expression vector
10 that includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, integration of the IAP-encoding gene into the host cell chromosome is selected for by inclusion of 0.01-300 μ M methotrexate in the cell culture medium (as described, Ausubel et al.,
15 *supra*). This dominant selection can be accomplished in most cell types. Recombinant protein expression can be increased by DHFR-mediated amplification of the transfected gene.

Methods for selecting cell lines bearing gene
20 amplifications are described in Ausubel et al. (*supra*). These methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. The most commonly used DHFR-containing expression vectors are pCVSEII-DHFR and pAdd26SV(A)
25 (described in Ausubel et al., *supra*). The host cells described above or, preferably, a DHFR-deficient CHO cell line (e.g., CHO DHFR⁻ cells, ATCC Accession No. CRL 9096) are among those most preferred for DHFR selection of a stably-transfected cell line or DHFR-mediated gene
30 amplification.

Once the recombinant protein is expressed, it is isolated by, for example, affinity chromatography. In one example, an anti-IAP antibody, which may be produced by the methods described herein, can be attached to a
35 column and used to isolate the IAP protein. Lysis and

- 38 -

fractionation of IAP-harboring cells prior to affinity chromatography may be performed by standard methods (see e.g., Ausubel et al., *supra*). Once isolated, the recombinant protein can, if desired, be purified further
5 by e.g., by high performance liquid chromatography (HPLC; e.g., see Fisher, Laboratory Techniques In Biochemistry And Molecular Biology, Work and Burdon, Eds., Elsevier, 1980).

Polypeptides of the invention, particularly
10 short IAP fragments, can also be produced by chemical synthesis (e.g., by the methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984 The Pierce Chemical Co., Rockford, IL). These general techniques of polypeptide expression and purification can also be used to produce
15 and isolate useful IAP fragments or analogs, as described herein.

VII. Anti-IAP Antibodies

In order to generate IAP-specific antibodies, an IAP coding sequence (i.e., amino acids 180-276) can be
20 expressed as a C-terminal fusion with glutathione S-transferase (GST; Smith et al., *Gene* 67:31-40, 1988). The fusion protein can be purified on glutathione-Sepharose beads, eluted with glutathione, and cleaved with thrombin (at the engineered cleavage site), and
25 purified to the degree required to successfully immunize rabbits. Primary immunizations can be carried out with Freund's complete adjuvant and subsequent immunizations performed with Freund's incomplete adjuvant. Antibody titres are monitored by Western blot and
30 immunoprecipitation analyses using the thrombin-cleaved IAP fragment of the GST-IAP fusion protein. Immune sera are affinity purified using CNBr-Sepharose-coupled IAP protein. Antiserum specificity is determined using a panel of unrelated GST proteins (including GSTp53, Rb,

- 39 -

HPV-16 E6, and E6-AP) and GST-trypsin (which was generated by PCR using known sequences).

As an alternate or adjunct immunogen to GST fusion proteins, peptides corresponding to relatively
5 unique hydrophilic regions of IAP may be generated and coupled to keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides is similarly affinity purified on peptides conjugated to BSA, and specificity is tested by ELISA and
10 Western blotting using peptide conjugates, and by Western blotting and immunoprecipitation using IAP expressed as a GST fusion protein.

Alternatively, monoclonal antibodies may be prepared using the IAP proteins described above and
15 standard hybridoma technology (see, e.g., Kohler et al., Nature 256:495, 1975; Kohler et al., Eur. J. Immunol. 6:511, 1976; Kohler et al., Eur. J. Immunol. 6:292, 1976; Hammerling et al., In Monoclonal Antibodies and T Cell Hybridomas,
20 Elsevier, New York, NY, 1981; Ausubel et al., *supra*). Once produced, monoclonal antibodies are also tested for specific IAP recognition by Western blot or immunoprecipitation analysis (by the methods described in Ausubel et al., *supra*).

25 Antibodies that specifically recognize IAPs or fragments of IAPs, such as those described herein containing one or more BIR domains (but not a ring zinc finger domain), or that contain a ring zinc finger domain (but not a BIR domain) are considered useful in the
30 invention. They may, for example, be used in an immunoassay to monitor IAP expression levels or to determine the subcellular location of an IAP or IAP fragment produced by a mammal. Antibodies that inhibit the 26 kDa IAP cleavage product described herein (which
35 contains at least one BIR domain) may be especially

- 40 -

useful in inducing apoptosis in cells undergoing undesirable proliferation.

Preferably, antibodies of the invention are produced using IAP sequence that does not reside within highly conserved regions, and that appears likely to be antigenic, as analyzed by criteria such as those provided by the Peptide structure program (Genetics Computer Group Sequence Analysis Package, Program Manual for the GCG Package, Version 7, 1991) using the algorithm of Jameson and Wolf (CABIOS 4:181, 1988). Specifically, these regions, which are found between BIR1 and BIR2 of all IAPs, are: from amino acid 99 to amino acid 170 of hiap-1, from amino acid 123 to amino acid 184 of hiap-2, and from amino acid 116 to amino acid 133 of either xiap or m-xiap. These fragments can be generated by standard techniques, e.g. by the PCR, and cloned into the pGEX expression vector (Ausubel et al., *supra*). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel et al. (*supra*). In order to minimize the potential for obtaining antisera that is non-specific, or exhibits low-affinity binding to IAP, two or three fusions are generated for each protein, and each fusion is injected into at least two rabbits. Antisera are raised by injections in series, preferably including at least three booster injections.

VIII. Identification of Molecules that Modulate IAP Protein Expression

Isolation of IAP cDNAs also facilitates the identification of molecules that increase or decrease IAP expression. In one approach, candidate molecules are added, in varying concentration, to the culture medium of cells expressing IAP mRNA. IAP expression is then measured, for example, by Northern blot analysis (Ausubel et al., *supra*) using an IAP cDNA, or cDNA fragment, as a hybridization probe (see also Table 5). The level of IAP

- 41 -

expression in the presence of the candidate molecule is compared to the level of IAP expression in the absence of the candidate molecule, all other factors (e.g. cell type and culture conditions) being equal.

5 The effect of candidate molecules on IAP-mediated apoptosis may, instead, be measured at the level of translation by using the general approach described above with standard protein detection techniques, such as Western blotting or immunoprecipitation with an IAP-specific antibody (for example, the IAP antibody
10 described herein).

Compounds that modulate the level of IAP may be purified, or substantially purified, or may be one component of a mixture of compounds such as an extract or
15 supernatant obtained from cells (Ausubel et al., *supra*). In an assay of a mixture of compounds, IAP expression is tested against progressively smaller subsets of the compound pool (e.g., produced by standard purification techniques such as HPLC or FPLC) until a single compound
20 or minimal number of effective compounds is demonstrated to modulate IAP expression.

Compounds may also be screened for their ability to modulate IAP apoptosis inhibiting activity. In this approach, the degree of apoptosis in the presence of a
25 candidate compound is compared to the degree of apoptosis in its absence, under equivalent conditions. Again, the screen may begin with a pool of candidate compounds, from which one or more useful modulator compounds are isolated in a step-wise fashion. Apoptosis activity may be
30 measured by any standard assay, for example, those described herein.

Another method for detecting compounds that modulate the activity of IAPs is to screen for compounds that interact physically with a given IAP polypeptide.
35 These compounds may be detected by adapting interaction

- 42 -

trap expression systems known in the art. These systems detect protein interactions using a transcriptional activation assay and are generally described by Gyuris et al. (Cell 75:791-803, 1993) and Field et al., Nature 5 340:245-246, 1989), and are commercially available from Clontech (Palo Alto, CA). In addition, PCT Publication WO 95/28497 describes an interaction trap assay in which proteins involved in apoptosis, by virtue of their interaction with Bcl-2, are detected. A similar method 10 may be used to identify proteins and other compounds that interact with IAPs.

Compounds or molecules that function as modulators of IAP-mediated cell death may include peptide and non-peptide molecules such as those present in cell 15 extracts, mammalian serum, or growth medium in which mammalian cells have been cultured.

A molecule that promotes an increase in IAP expression or IAP activity is considered particularly useful in the invention; such a molecule may be used, for 20 example, as a therapeutic to increase cellular levels of IAP and thereby exploit the ability of IAP polypeptides to inhibit apoptosis.

A molecule that decreases IAP activity (e.g., by decreasing IAP gene expression or polypeptide activity) 25 may be used to decrease cellular proliferation. This would be advantageous in the treatment of neoplasms (see Table 3, below), or other cell proliferative diseases.

- 43 -

TABLE 3

NORTHERN BLOT IAP RNA LEVELS IN CANCER CELLS*

	xiap	hiap1	hiap2
Promyelocytic Leukemia HL-60	+	+	+
Hela S-3	+	+	+
5 Chronic Myelogenous Leukemia K-562	+++	+	+++
Lymphoblastic Leukemia MOLT-4	+++	+	+
Burkitt's Lymphoma Raji	+	+(x10)	+
Colorectal Adenocarcinoma SW-480	+++	+++	+++
Lung Carcinoma A-549	+	+	+
10 Melanoma G-361	+++	+	+

*Levels are indicated by a (+) and are the approximate increase in RNA levels relative to Northern blots of RNA from non-cancerous control cell lines. A single plus indicates an estimated increase of at least 1-fold

15 Molecules that are found, by the methods described above, to effectively modulate IAP gene expression or polypeptide activity may be tested further in animal models. If they continue to function successfully in an *in vivo* setting, they may be used as
20 therapeutics to either inhibit or enhance apoptosis, as appropriate.

IX. IAP Therapy

The level of IAP gene expression correlates with the level of apoptosis. Thus, IAP genes also find use in
25 anti-apoptosis gene therapy. In particular, a functional IAP gene may be used to sustain neuronal cells that undergo apoptosis in the course of a neurodegenerative disease, lymphocytes (i.e., T cells and B cells), or cells that have been injured by ischemia.

30 Retroviral vectors, adenoviral vectors, adeno-associated viral vectors, or other viral vectors with the appropriate tropism for cells likely to be involved in

- 44 -

apoptosis (for example, epithelial cells) may be used as a gene transfer delivery system for a therapeutic IAP gene construct. Numerous vectors useful for this purpose are generally known (Miller, Human Gene Therapy 15-14, 5 1990; Friedman, Science 244:1275-1281, 1989; Eglitis and Anderson, BioTechniques 6:608-614, 1988; Tolstoshev and Anderson, current opinion in Biotechnology 1:55-61, 1990; Sharp, The Lancet 337:1277-1278, 1991; Cornetta et al., Nucleic Acid Research and Molecular Biology 36:311-322, 10 1987; Anderson, Science 226:401-409, 1984; Moen, Blood Cells 17:407-416, 1991; Miller et al., Biotechniques 7:980-990, 1989; Le Gal La Salle et al., Science 259:988-990, 1993; and Johnson, Chest 107:77S-83S, 1995).

Retroviral vectors are particularly well developed and 15 have been used in clinical settings (Rosenberg et al., N. Engl. J. Med 323:370, 1990; Anderson et al., U.S. Patent No. 5,399,346). Non-viral approaches may also be employed for the introduction of therapeutic DNA into cells otherwise predicted to undergo apoptosis. For 20 example, IAP may be introduced into a neuron or a T cell by lipofection (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413, 1987; Ono et al., Neurosci. Lett. 117:259, 1990; Brigham et al., Am. J. Med. Sci. 298:278, 1989; Staubinger et al., Meth. Enz. 101:512, 1983), 25 asialorosonucoid-polylysine conjugation (Wu et al., J. Biol. Chem. 263:14621, 1988; Wu et al., J. Biol. Chem. 264:16985, 1989); or, less preferably, microinjection under surgical conditions (Wolff et al., Science 247:1465, 1990).

30 For any of the methods of application described above, the therapeutic IAP DNA construct is preferably applied to the site of the predicted apoptosis event (for example, by injection). However, it may also be applied to tissue in the vicinity of the predicted apoptosis

- 45 -

event or to a blood vessel supplying the cells predicted to undergo apoptosis.

In the constructs described, IAP cDNA expression can be directed from any suitable promoter (e.g., the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein promoters), and regulated by any appropriate mammalian regulatory element. For example, if desired, enhancers known to preferentially direct gene expression in neural cells, T cells, or B cells may be used to direct IAP expression. The enhancers used could include, without limitation, those that are characterized as tissue- or cell-specific in their expression. Alternatively, if an IAP genomic clone is used as a therapeutic construct (for example, following its isolation by hybridization with the IAP cDNA described above), regulation may be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.

Less preferably, IAP gene therapy is accomplished by direct administration of the IAP mRNA or antisense IAP mRNA to a cell that is expected to undergo apoptosis. The mRNA may be produced and isolated by any standard technique, but is most readily produced by *in vitro* transcription using an IAP cDNA under the control of a high efficiency promoter (e.g., the T7 promoter). Administration of IAP mRNA to malignant cells can be carried out by any of the methods for direct nucleic acid administration described above.

Ideally, the production of IAP protein by any gene therapy approach will result in cellular levels of IAP that are at least equivalent to the normal, cellular level of IAP in an unaffected cell. Treatment by any

- 46 -

IAP-mediated gene therapy approach may be combined with more traditional therapies.

Another therapeutic approach within the invention involves administration of recombinant IAP protein, either directly to the site of a predicted apoptosis event (for example, by injection) or systemically (for example, by any conventional recombinant protein administration technique). The dosage of IAP depends on a number of factors, including the size and health of the individual patient, but, generally, between 0.1 mg and 100 mg inclusive are administered per day to an adult in any pharmaceutically-acceptable formulation.

X. Administration of IAP Polypeptides, IAP Genes, or Modulators of IAP Synthesis or Function

An IAP protein, gene, or modulator may be administered within a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer IAP to patients suffering from a disease that is caused by excessive apoptosis. Administration may begin before the patient is symptomatic. Any appropriate route of administration may be employed, for example, administration may be parenteral, intravenous, intraarterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found, for example, in "Remington's

- 47 -

Pharmaceutical Sciences." Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or
5 hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for IAP
10 modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example,
15 polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

If desired, treatment with an IAP protein, gene, or modulatory compound may be combined with more
20 traditional therapies for the disease such as surgery, steroid therapy, or chemotherapy for autoimmune disease; antiviral therapy for AIDS; and tissue plasminogen activator (TPA) for ischemic injury.

25 XI. Detection of Conditions Involving Altered Apoptosis

IAP polypeptides and nucleic acid sequences find diagnostic use in the detection or monitoring of conditions involving aberrant levels of apoptosis. For example, decrease expression of IAP may be correlated
30 with enhanced apoptosis in humans (see XII, below). Accordingly, a decrease or increase in the level of IAP production may provide an indication of a deleterious condition. Levels of IAP expression may be assayed by any standard technique. For example, IAP expression in a
35 biological sample (e.g., a biopsy) may be monitored by standard Northern blot analysis or may be aided by PCR

- 48 -

(see, e.g., Ausubel et al., *supra*; PCR Technology: Principles and Applications for DNA Amplification, H.A. Ehrlich, Ed. Stockton Press, NY; Yap et al. Nucl. Acids. Res. 19:4294, 1991).

5 Alternatively, a biological sample obtained from a patient may be analyzed for one or more mutations in the IAP sequences using a mismatch detection approach. Generally, these techniques involve PCR amplification of nucleic acid from the patient sample, followed by
10 identification of the mutation (i.e., mismatch) by either altered hybridization, aberrant electrophoretic gel migration, binding or cleavage mediated by mismatch binding proteins, or direct nucleic acid sequencing. Any of these techniques may be used to facilitate mutant IAP
15 detection, and each is well known in the art; examples of particular techniques are described, without limitation, in Orita et al., Proc. Natl. Acad. Sci. USA 86:2766-2770, 1989; Sheffield et al., Proc. Natl. Acad. Sci. USA 86:232-236, 1989).

20 In yet another approach, immunoassays are used to detect or monitor IAP protein in a biological sample. IAPspecific polyclonal or monoclonal antibodies (produced as described above) may be used in any standard immunoassay format (e.g., ELISA, Western blot, or RIA) to
25 measure IAP polypeptide levels. These levels would be compared to wild-type IAP levels, with a decrease in IAP production indicating a condition involving increased apoptosis. Examples of immunoassays are described, e.g., in Ausubel et al., *supra*. Immunohistochemical techniques
30 may also be utilized for IAP detection. For example, a tissue sample may be obtained from a patient, sectioned, and stained for the presence of IAP using an anti-IAP antibody and any standard detection system (e.g., one which includes a secondary antibody conjugated to
35 horseradish peroxidase). General guidance regarding such

- 49 -

techniques can be found in, e.g., Bancroft and Stevens
(Theory and Practice of Histological Techniques,
Churchill Livingstone, 1982) and Ausubel et al. (*supra*).

In one preferred example, a combined diagnostic
5 method may be employed that begins with an evaluation of
IAP protein production (for example, by immunological
techniques or the protein truncation test (Hogerrorst et
al., Nature Genetics 10:208-212, 1995) and also includes
a nucleic acid-based detection technique designed to
10 identify more subtle IAP mutations (for example, point
mutations). As described above, a number of mismatch
detection assays are available to those skilled in the
art, and any preferred technique may be used. Mutations
in IAP may be detected that either result in loss of IAP
15 expression or loss of IAP biological activity. In a
variation of this combined diagnostic method, IAP
biological activity is measured as protease activity
using any appropriate protease assay system (for example,
those described above).

20 Mismatch detection assays also provide an
opportunity to diagnose an IAP-mediated predisposition to
diseases caused by inappropriate apoptosis. For example,
a patient heterozygous for an IAP mutation may show no
clinical symptoms and yet possess a higher than normal
25 probability of developing one or more types of
neurodegenerative, myelodysplastic or ischemic diseases.
Given this diagnosis, a patient may take precautions to
minimize their exposure to adverse environmental factors
(for example, UV exposure or chemical mutagens) and to
30 carefully monitor their medical condition (for example,
through frequent physical examinations). This type of
IAP diagnostic approach may also be used to detect IAP
mutations in prenatal screens. The IAP diagnostic assays
described above may be carried out using any biological
35 sample (for example, any biopsy sample or bodily fluid or

- 50 -

tissue) in which IAP is normally expressed. Identification of a mutant IAP gene may also be assayed using these sources for test samples.

Alternatively, a IAP mutation, particularly as
5 part of a diagnosis for predisposition to IAP-associated degenerative disease, may be tested using a DNA sample from any cell, for example, by mismatch detection techniques. Preferably, the DNA sample is subjected to PCR amplification prior to analysis.

10 In order to demonstrate the utility of IAP gene sequences as diagnostics and prognostics for cancer, a Human Cancer Cell Line Multiple Tissue Northern Blot (Clontech, Palo Alto, CA; #7757-1) was probed. This Northern blot contained approximately 2 μ g of poly A⁺ RNA
15 per lane from eight different human cell lines: (1) promyelocytic leukemia HL-60, (2) HeLa cell S3, (3) chronic myelogenous leukemia K-562, (4) lymphoblastic leukemia MOLT-4, (5) Burkitt's lymphoma Raji, (6) colorectal adenocarcinoma SW480, (7) lung carcinoma A549,
20 and (8) melanoma G361. As a control, a Human Multiple Tissue Northern Blot (Clontech, Palo Alto, CA; #7759-1) was probed. This Northern blot contained approximately 2 μ g of poly A⁺ RNA from eight different human tissues: (1) spleen, (2) thymus, (3) prostate, (4) testis, (5)
25 ovary, (6) small intestine, (7) colon, and (8) peripheral blood leukocytes.

The Northern blots were hybridized sequentially with: (1) a 1.6 kb probe to the xiap coding region, (2) a 375 bp hiap-2 specific probe corresponding to the
30 3' untranslated region, (3) a 1.3 kb probe to the coding region of hiap-1, which cross-reacts with hiap-2, (4) a 1.0 kb probe derived from the coding region of bcl-2, and (5) a probe to β -actin, which was provided by the manufacturer. Hybridization was carried out at 50°C
35 overnight, according to the manufacturer's suggestion.

- 51 -

The blot was washed twice with 2X SSC, 0.1% SDS at room temperature for 15 minutes and then with 2X SSC, 0.1% SDS at 50°C.

All cancer lines tested showed increased IAP expression relative to samples from non-cancerous control tissues (Table 3). Expression of xiap was particularly high in HeLa (S-3), chronic myelogenous leukemia (K-562), colorectal adenocarcinoma (SW-480), and melanoma (G-361) lines. Expression of hiap-1 was extremely high in Burkitt's lymphoma, and was also elevated in colorectal adenocarcinoma. Expression of hiap-2 was particularly high in chronic myelogenous leukemia (K-562) and colorectal adenocarcinoma (SW-480). Expression of Bcl-2 was upregulated only in HL-60 leukemia cells.

These observations suggest that upregulation of the anti-apoptotic IAP genes may be a widespread phenomenon, perhaps occurring much more frequently than upregulation of Bcl-2. Furthermore, upregulation may be necessary for the establishment or maintenance of the transformed state of cancerous cells.

In order to pursue the observation described above, i.e., that hiap-1 is overexpressed in the Raji Burkitt's lymphoma cell line, RT-PCR analysis was performed in multiple Burkitt's lymphoma cell lines. Total RNA was extracted from cells of the Raji, Ramos, EB-3, and Jiyoye cell lines, and as a positive control, from normal placental tissue. The RNA was reverse transcribed, and amplified by PCR with the following set of oligonucleotide primers:

5'-AGTGCGGGTTTTATTATGTG-3' (SEQ ID NO:__) and
5'-AGATGACCACAAGGAATAAACAATA-3' (SEQ ID NO:__), which selectively amplify a hiap-1 cDNA fragment. RT-PCR was conducted using a PerkinElmer 480 Thermocycler to carry out 35 cycles of the following program: 94°C for 1 minute, 50°C for 1.5 minutes, and 72°C for a minute. The

- 52 -

PCR reaction product was electrophoresed on an agarose gel and stained with Ethidium bromide. Amplified cDNA fragments of the appropriate size were clearly visible in all lanes containing Burkitt's lymphoma samples, but
5 absent in the lanes containing the normal placental tissue sample, and absent in lanes containing negative control samples, where template DNA was omitted from the reaction (Fig. 17).

XII. Accumulation of a 26 kDa
Cleavage Protein in Astrocytoma Cells

10

A. Identification of a 26 kDa Cleavage Protein

A total protein extract was prepared from Jurkat and astrocytoma cells by sonicating them (X3 for 15 seconds at 4°C) in 50 mM Tris-HCl (pH 8.0), 150 mM NaCl,
15 1 mM PMSF, 1 µg/ml aprotinin, and 5 mM benzamidine. Following sonication, the samples were centrifuged (14,000 RPM in a microfuge) for five minutes. Twenty µg of protein was loaded per well on a 10% SDS-polyacrylamide gel, electrophoresed, and electroblotted
20 by standard methods to PVDF membranes. Western blot analysis, performed as described previously, revealed that the astrocytoma cell line (CCF-STTG1) abundantly expressed an anti-xiap reactive band of approximately 26 kDa, despite the lack of an apoptotic trigger event (Fig.
25 18). In fact, this cell line has been previously characterized as being particularly resistant to standard apoptotic triggers.

A 26 kDa xiap-reactive band was also observed under the following experimental conditions. Jurkat
30 cells (a transformed human T cell line) were induced to undergo apoptosis by exposure to an anti-Fas antibody (1 µg/ml). Identical cultures of Jurkat cells were exposed either to: (1) anti-Fas antibody and cycloheximide (20 µg/ml), (2) tumor necrosis factor alpha (TNF-α, at 1,000
35 U/ml), or (3) TNF-α and cycloheximide (20 µg/ml). All cells were harvested 6 hours after treatment began. In

- 53. -

addition, as a negative control, anti-Fas antibody was added to an extract after the cells were harvested. The cells were harvested in SDS sample buffer, electrophoresed on a 12.5% SDS polyacrylamide gel, and electroblotted onto PVDF membranes using standard methods. The membranes were immunostained with a rabbit polyclonal anti-XIAP antibody at 1:1000 for 1 hour at room temperature. Following four 15 minute washes, a goat anti-rabbit antibody conjugated to horse-radish peroxidase was applied at room temperature for 1 hour. Unbound secondary antibody was washed away, and chemiluminescent detection of XIAP protein was performed. The Western blot revealed the presence of the full-length, 55 kDa XIAP protein, both in untreated and treated cells. In addition, a novel, approximately 26 kDa xiap-reactive band was also observed in apoptotic cell extracts, but not in the control, untreated cell extracts (Fig. 19).

Cleavage of XIAP occurs in a variety of cell types, including other cancer cell lines such as HeLa. The expression of the 26 kDa XIAP cleavage product was demonstrated in HeLa cells as follows. HeLa cells were treated with either: (1) cyclohexamide (20 μ g/ml), (2) anti-Fas antibody (1 μ g/ml), (3) anti-Fas antibody (1 μ g/ml) and cyclohexamide (20 μ g/ml), (4) TNF α (1,000 U/ml), or (5) TNF α (1,000 U/ml) and cyclohexamide (20 μ g/ml). All cells were harvested 18 hours after treatment began. As above, anti-Fas antibody was added to an extract after the cells were harvested. HeLa cells were harvested, and the Western blot was probed under the same conditions as used to visualize xiap-reactive bands from Jurkat cell samples. A 26 kDa XIAP band was again seen in the apoptotic cell preparations (Fig. 20). Furthermore, the degree of XIAP cleavage correlated positively with the extent of apoptosis. Treatment of

- 54 -

HeLa cells with cycloheximide or TNF α alone caused only minor apoptosis, and little cleavage product was observed. If the cells were treated with the anti-Fas antibody, a greater amount of cleavage product was
5 apparent. These data indicate that XIAP is cleaved in more than one cell type and in response to more than one type of apoptotic trigger.

B. Time Course of Expression

The time course over which the 26 kDa cleavage
10 product accumulates was examined by treating HeLa and Jurkat cells with anti-Fas antibody (1 μ g/ml) and harvesting them either immediately, or 1, 2, 3, 5, 10, or 22 hours after treatment. Protein extracts were prepared and Western blot analysis was performed as described
15 above. Both types of cells accumulated increasing quantities of the 26 kDa cleavage product over the time course examined (Figs. 21A and 21B).

C. Subcellular Localization of the 26 kDa XIAP Cleavage Product

20 In order to determine the subcellular location of the 26 kDa cleavage product, Jurkat cells were induced to undergo apoptosis by exposure to anti-Fas antibody (1 μ g/ml) and were then harvested either immediately, 3 hours, or 7 hours later. Total protein extracts were
25 prepared, as described above, from cells harvested at each time point. In order to prepare nuclear and cytoplasmic cell extracts, apoptotic Jurkat cells were washed with isotonic Tris buffered saline (pH 7.0) and lysed by freezing and thawing five times in cell
30 extraction buffer (50 mM PIPES, 50 mM KCl, 5 mM EGTA, 2 mM MgCl₂, 1 mM DTT, and 20 μ M cytochalasin B). Nuclei were pelleted by centrifugation and resuspended in isotonic Tris (pH 7.0) and frozen at -80°C. The
35 cytoplasmic fraction of the extract was processed further by centrifugation at 60,000 RPM in a TA 100.3 rotor for 30 minutes. Supernatants were removed and frozen at -

- 55 -

80°C. Samples of both nuclear and cytoplasmic fractions were loaded on a 12.5% SDS-polyacrylamide gel, and electroblotted onto PVDF membranes. Western blot analysis was then performed using either an anti-CPP32 antibody (Transduction Laboratories Lexington, KY; Fig. 22A) or the rabbit anti-XIAP antibody described above (Fig. 22B).

The anti-CPP32 antibody, which recognizes the CPP32 protease (also known as YAMA or Apopain) partitioned almost exclusively in the cytoplasmic fraction. The 55 kDa XIAP protein localized exclusively in the cytoplasm of apoptotic cells, in agreement with the studies presented above, where XIAP protein in normal, healthy COS cells was seen to localize, by immunofluorescence microscopy, to the cytoplasm. In contrast, the 26 kDa cleavage product localized exclusively to the nuclear fraction of apoptotic Jurkat cells. Taken together, these observations suggest that the anti-apoptotic component of XIAP could be the 26 kDa cleavage product, which exerts its influence within the nucleus.

D. In vitro Cleavage of XIAP protein and Characterization of the Cleavage Product

For this series of experiments, XIAP protein was labeled with ³⁵S using the plasmid pcDNA3-6myc-XIAP, T7 RNA polymerase, and a coupled transcription/translation kit (Promega) according to the manufacturer's instructions. Radioactively labeled XIAP protein was separated from unincorporated methionine by column chromatography using Sephadex G-50™. In addition, extracts of apoptotic Jurkat cells were prepared following treatment with anti-Fas antibody (1 µg/ml) for three hours. To prepare the extracts, the cells were lysed in Triton X-100 buffer (1% Triton X-100, 25 mM Tris HCl) on ice for two hours and then microcentrifuged for 5 minutes. The soluble extract was retained (and was

- 56 -

labeled TX100). Cells were lysed in cell extraction buffer with freeze/thawing. The soluble cytoplasmic fraction was set aside (and labeled CEB). Nuclear pellets from the preparation of the CEB cytoplasmic
5 fraction were solubilized with Triton X-100 buffer, microcentrifuged, and the soluble fractions, which contains primarily nuclear DNA, was retained (and labeled CEB-TX100). Soluble cell extract was prepared by lysing cells with NP-40 buffer, followed by microcentrifugation
10 for 5 minutes (and was labeled NP-40). *In vitro* cleavage was performed by incubating 16 μ l of each extract (CEB, TX-100, CEB-TX100, and NP-40) with 4 μ l of *in vitro* translated XIAP protein at 37°C for 7 hours. Negative controls, containing only TX100 buffer or CEB buffer were
15 also included. The proteins were separated on a 10% SDS-polyacrylamide gel, which was dried and exposed to X-ray film overnight.

In vitro cleavage of XIAP was apparent in the CEB extract. The observed molecular weight of the
20 cleavage product was approximately 36 kDa (Fig. 23). The 10 kDa shift in the size of the cleavage product indicates that the observed product is derived from the amino-terminus of the recombinant protein, which contains six copies of the myc epitope (10 kDa). It thus appears
25 that the cleavage product possesses at least two of the BIR domains, and that it is localized to the nucleus.

XIII. Treatment of HIV Infected Individuals

The expression of hiap-1 and hiap-2 is decreased significantly in HIV-infected human cells. Furthermore,
30 this decrease precedes apoptosis. Therefore, administration of HIAP-1, HIAP-2, genes encoding these proteins, or compounds that upregulate these genes can be used to prevent T cell attrition in HIV-infected patients. The following assay may also be used to screen

- 57 -

for compounds that alter hiap-1 and hiap-2 expression, and which also prevent apoptosis.

Cultured mature lymphocyte CD-4⁺ T cell lines (H9, labelled "a"; CEM/CM-3, labelled "b"; 6T-CEM, labelled "c"; and Jurkat, labelled "d" in Figs. 13A and 13B), were examined for signs of apoptosis (Fig. 13A) and hiap gene expression (Fig. 13B) after exposure to mitogens or HIV infection. Apoptosis was demonstrated by the appearance of DNA "laddering" upon gel electrophoresis and gene expression was assessed by PCR. The results obtained from normal (non-infected, non-mitogen stimulated) cells are shown in each lane labelled "1" in Figs. 13A and 13B. The results obtained 24 hours after PHA/PMA (phytohemagglutinin/phorbol ester) stimulation are shown in each lane labelled "2". The results obtained 24 hours after HIV strain III_B infection are shown in each lane labelled "3". The "M" refers to standard DNA markers (the 123 bp ladder in Fig. 13B, and the lambda HindIII ladder in Fig. 13A (both from Gibco-BRL)). DNA ladders (Prigent et al., J. Immunol. Methods, 160:139-140, 1993), which indicate apoptosis, are evident when DNA from the samples described above are electrophoresed on an ethidium bromide-stained agarose gel (Fig. 13A). The sensitivity and degree of apoptosis of the four T cell lines tested varies following mitogen stimulation and HIV infection.

In order to examine hiap gene expression, total RNA was prepared from the cultured cells and reverse transcribed using oligo-dT priming. The RT cDNA products were amplified by PCR using specific primers (as shown in Table 5) for the detection of hiap-2a, hiap-2b and hiap-1. The PCR was conducted using a PerkinElmer 480 thermocycler with 35 cycles of the following program: 94°C for one minute, 55°C for 2 minutes and 72°C for 1.5 minutes. The RT-PCR reaction products were

- 58 -

electrophoresed on a 1% agarose gel, which was stained with ethidium bromide. Absence of hiap-2 transcripts is noted in all four cell lines 24 hours after HIV infection. In three of four cell lines (all except H9), the hiap-1 gene is also dramatically down-regulated after HIV infection. PHA/PMA mitogen stimulation also appears to decrease hiap gene expression; particularly of hiap-2 and to a lesser extent, of hiap-1. The data from these experiments is summarized in Table 5. The expression of β -actin was consistent in all cell lines tested, indicating that there is not a flaw in the RT-PCR assay that could account for the decrease in hiap gene expression.

TABLE 4

OLIGONUCLEOTIDE PRIMERS FOR THE SPECIFIC RT-PCR AMPLIFICATION OF UNIQUE IAP GENES

IAP Gene	Forward Primer (nucleotide position*)	Reverse Primer (nucleotide position*)	Size of Product (bp)
h-xiap	p2415 (876-896)	p2449 (1291-1311)	435
m-xiap	p2566 (458-478)	p2490 (994-1013)	555
h-hiap1	p2465 (827-847)	p2464 (1008-1038)	211
m-hiap1	p2687 (747-767)	p2684 (1177-1197)	450
hiap2	p2595 (1562-1585)	p2578 (2339-2363)	801 ^a 618 ^b
m-hiap2	p2693 (1751-1772)	p2734 (2078-2100)	349

* Nucleotide position as determined from Figs. 1-4 for each

IAP gene

^a PCR product size of hiap2a

^b PCR product size of hiap2b

- 59 -

TABLE 5

APOPTOSIS AND HIAP GENE EXPRESSION IN CULTURED T-CELLS
FOLLOWING MITOGEN STIMULATION OR HIV INFECTION

Cell Line	Condition	Apoptosis	hiap1	hiap2
5 H9	not stimulated	-	+	±
	PHA/PMA stimulated	+++	+	±
	HIV infected	++	+	-
CEM/CM-3	not stimulated	-	+	±
	PHA/PMA stimulated	±	+	-
	HIV infected	±	-	-
6T-CEM	not stimulated	-	+	+
	PHA/PMA stimulated	±	-	-
	HIV infected	+	-	-
Jurkat	not stimulated	-	+	++
	PHA/PMA stimulated	+	+	+
	HIV infected	±	-	-

XIV. Assignment of xiap, hiap-1, and hiap-2 to
Chromosomes Xq25 and 11q22-23 by
Fluorescence in situ Hybridization

(FISH)

5 Fluorescence in situ hybridization (FISH) was
used to identify the chromosomal location of xiap, hiap-1
and hiap-2. The probes used were cDNAs cloned in plasmid
vectors: the 2.4 kb xiap clone included 1493 bp of
coding sequence, 34bp of 5' UTR (untranslated region) and
10 913 bp of 3'UTR; the hiap-1 cDNA was 3.1 kb long and
included 1812 bp coding and 1300 bp of 3' UTR; and the
hiap-2 clone consisted of 1856 bp of coding and 1200 bp
of 5' UTR. A total of 1 µg of probe DNA was labelled
with biotin by nick translation (BRL). Chromosome
15 spreads prepared from a normal peripheral blood culture
were denatured for 2 minutes at 70°C in 50% formamide/2X
SSC and subsequently hybridized with the biotin labelled
DNA probe for 18 hours at 37°C in a solution consisting
of 2X SSC/70% formamide/10% dextran sulfate. After
20 hybridization, the spreads were washed in
2X SSC/50% formamide, followed by a wash in 2X SSC at
42°C. The biotin labelled DNA was detected by
fluorescein isothiocyanate (FITC) conjugated avidin
antibodies and anti-avidin antibodies (ONCOR detection
25 kit), according to the manufacturer's instructions.
Chromosomes were counterstained with propidium iodide and
examined with a Olympus BX60 epifluorescence microscope.
For chromosome identification, the slides with recorded
labelled metaphase spreads were destained, dehydrated,
30 dried, digested with trypsin for 30 seconds and stained
with 4% Giemsa stain for 2 minutes. The chromosome
spreads were relocated and the images were compared.

A total of 101 metaphase spreads were examined
with the xiap probe, as described above. Symmetrical
35 fluorescent signals on either one or both homologs of

- 61 -

chromosome Xq25 were observed in 74% of the cells analyzed. Following staining with hiap-1 and hiap-2 probes, 56 cells were analyzed and doublet signals in the region 11q22-23 were observed in 83% of cells examined.

5 The xiap gene was mapped to Xq25 while the hiap-1 and hiap-2 genes were mapped at the border of 11q22 and 11q23 bands.

These experiments confirmed the location of the xiap gene on chromosome Xq25. No highly consistent

10 chromosomal abnormalities involving band Xq25 have been reported so far in any malignancies. However, deletions within this region are associated with a number of immune system defects including X-linked lymphoproliferative disease (Wu et al., Genomics 17:163-170, 1993).

15 Cytogenetic abnormalities of band 11q23 have been identified in more than 50% of infant leukemias regardless of the phenotype (Martinez-Climet et al., Leukaemia 9:1299-1304, 1995). Rearrangements of the MLL Gene (mixed lineage leukemia or myeloid lymphoid

20 leukemia; Ziemer Van der Poel et al., Proc. Natl. Acad. Sci. USA 88:10735-10739, 1991) have been detected in 80% of cases with 11q23 translocation, however patients whose rearrangements clearly involved regions other than the MLL gene were also reported (Kobayashi et al., Blood

25 82:547-551, 1993). Thus, the IAP genes may follow the Bcl-2 paradigm, and would therefore play an important role in cancer transformation.

XV. Preventive Anti-Apoptotic Therapy

In a patient diagnosed to be heterozygous for an

30 IAP mutation or to be susceptible to IAP mutations (even if those mutations do not yet result in alteration or loss of IAP biological activity), or a patient diagnosed as HIV positive, any of the above therapies may be administered before the occurrence of the disease

- 62 -

phenotype. For example, the therapies may be provided to a patient who is HIV positive but does not yet show a diminished T cell count or other overt signs of AIDS. In particular, compounds shown to increase IAP expression or IAP biological activity may be administered by any standard dosage and route of administration (see above). Alternatively, gene therapy using an IAP expression construct may be undertaken to reverse or prevent the cell defect prior to the development of the degenerative disease.

The methods of the instant invention may be used to reduce or diagnose the disorders described herein in any mammal, for example, humans, domestic pets, or livestock. Where a non-human mammal is treated or diagnosed, the IAP polypeptide, nucleic acid, or antibody employed is preferably specific for that species.

Other Embodiments

In other embodiments, the invention includes any protein which is substantially identical to a mammalian IAP polypeptides (Figs. 1-6; SEQ ID NOS:1-42); such homologs include other substantially pure naturally-occurring mammalian IAP proteins as well as allelic variants; natural mutants; induced mutants; DNA sequences which encode proteins and also hybridize to the IAP DNA sequences of Figs. 1-6 (SEQ ID NOS:1-42) under high stringency conditions or, less preferably, under low stringency conditions (e.g., washing at 2X SSC at 400C with a probe length of at least 40 nucleotides); and proteins specifically bound by antisera directed to a IAP polypeptide. The term also includes chimeric polypeptides that include a IAP portion.

The invention further includes analogs of any naturally-occurring IAP polypeptide. Analogs can differ from the naturally-occurring IAP protein by amino acid

- 63 -

sequence differences, by post-translational modifications, or by both. Analogs of the invention will generally exhibit at least 85%, more preferably 90%, and most preferably 95% or even 99% identity with all or part
5 of a naturally occurring IAP amino acid sequence. The length of sequence comparison is at least 15 amino acid residues, preferably at least 25 amino acid residues, and more preferably more than 35 amino acid residues. Modifications include *in vivo* and *in vitro* chemical
10 derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-
15 occurring IAP polypeptide by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to
ethanemethylsulfate or by site-specific mutagenesis as
20 described in Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual* (2d ed.), CSH Press, 1989, or Ausubel et al., *supra*). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, e.g., D-amino acids or non-
25 naturally occurring or synthetic amino acids, e.g., B or y amino acids. In addition to full-length polypeptides, the invention also includes IAP polypeptide fragments. As used herein, the term "fragment," means at least 20
contiguous amino acids, preferably at least 30 contiguous
30 amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least 60 to 80 or more contiguous amino acids. Fragments of IAP polypeptides can be generated by methods known to those skilled in the art or may result from normal protein processing (e.g.,
35 removal of amino acids from the nascent polypeptide that

- 64 -

are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events).

Preferable fragments or analogs according to the invention are those which facilitate specific detection of a IAP nucleic acid or amino acid sequence in a sample to be diagnosed. Particularly useful IAP fragments for this purpose include, without limitation, the amino acid fragments shown in Table 2.

10 What is claimed is:

- 65 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: University of Ottawa
Korneluk, Robert G.
Mackenzie, Alexander E.
Baird, Stephen
Liston, Peter
- (ii) TITLE OF INVENTION: MAMMALIAN IAP GENE FAMILY, PRIMERS,
PROBES, AND DETECTION METHODS
- (iii) NUMBER OF SEQUENCES: 45
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Fish & Richardson P.C.
 - (B) STREET: 225 Franklin Street
 - (C) CITY: Boston
 - (D) STATE: MA
 - (E) COUNTRY: USA
 - (F) ZIP: 02110-2804
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: PCT/IB96/----
 - (B) FILING DATE: 05-AUG-1996
 - (C) CLASSIFICATION:
- (vii) PRIORITY APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/511,485
 - (B) FILING DATE: 04-AUG-1995
 - (C) CLASSIFICATION:
- (vii) PRIORITY APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/576,956
 - (B) FILING DATE: 22-DEC-1995
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Clark, Paul T.
 - (B) REGISTRATION NUMBER: 30,162
 - (C) REFERENCE/DOCKET NUMBER: 07891/003WO1
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 617/542-5070
 - (B) TELEFAX: 617/542-8906
 - (C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

- 66 -

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 46 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa at positons 2, 3, 4, 5, 6, 7, 9, 10, 11, 17, 18, 19, 20, 21, 23, 25, 30, 31, 32, 34, 35, 38, 39, 40, 41, 42, and 45 may be any amino acid. Xaa at positon 8 is Glu or Asp. Xaa at positions 14 & 22 is Val or Ile.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

Glu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Lys Xaa Cys Met
1           5           10           15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Phe Xaa Pro Cys Gly His Xaa
Xaa Xaa           20           25           30
Cys Xaa Xaa Cys Ala Xaa Xaa Xaa Xaa Xaa Cys Pro Xaa Cys
35           40           45

```

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 68 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa at positions 1, 2, 3, 6, 9, 10, 14, 15, 18, 19, 20, 21, 24, 30, 32, 33, 35, 37, 40, 42, 43, 44, 45, 46, 47, 49, 50, 51, 53, 54, 55, 56, 57, 59, 60, 61, 62, 64 and 66 may be any amino acid. Xaa at positions 13, 16 and 17 may be any amino acid or may be absent.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Xaa Xaa Xaa Arg Leu Xaa Thr Phe Xaa Xaa Trp Pro Xaa Xaa Xaa Xaa
1           5           10           15
Xaa Xaa Xaa Xaa Xaa Leu Ala Xaa Ala Gly Phe Tyr Tyr Xaa Gly Xaa
20           25           30
Xaa Asp Xaa Val Xaa Cys Phe Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Trp
35           40           45
Xaa Xaa Xaa Asp Xaa Xaa Xaa Xaa Xaa His Xaa Xaa Xaa Xaa Pro Xaa
50           55           60

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- 67 -

Cys Xaa Phe Val
65

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2540 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAAAAGGTGG ACAAGTCCTA TTTTCAAGAG AAGATGACTT TTAACAGTTT TGAAGGATCT	60
AAAACTTGTG TACCTGCAGA CATCAATAAG GAAGAAGAAT TTGTAGAAGA GTTTAATAGA	120
TTAAAACTT TTGCTAATTT TCCAAGTGGT AGTCCTGTTT CAGCATCAAC ACTGGCACGA	180
GCAGGGTTTC TTTATACTGG TGAAGGAGAT ACCGTGCGGT GCTTTAGTTG TCATGCAGCT	240
GTAGATAGAT GGCAATATGG AGACTCAGCA GTTGGAAGAC ACAGGAAAAGT ATCCCCAAAT	300
TGCAGATTTA TCAACGGCTT TTATCTTGAA AATAGTGCCA CGCAGTCTAC AAATTCTGGT	360
ATCCAGAATG GTCAGTACAA AGTTGAAAAC TATCTGGGAA GCAGAGATCA TTTTGCCTTA	420
GACAGGCCAT CTGAGACACA TGCAGACTAT CTTTTGAGAA CTGGGCAGGT TGTAGATATA	480
TCAGACACCA TATACCCGAG GAACCCTGCC ATGTATTGTG AAGAAGCTAG ATTAAAGTCC	540
TTTCAGAACT GGCCAGACTA TGCTCACCTA ACCCCAAGAG AGTTAGCAAG TGCTGGACTC	600
TACTACACAG GTATTGGTGA CCAAGTGCAG TGCTTTTGTT GTGGTGAAA ACTGAAAAAT	660
TGGGAACCTT GTGATCGTGC CTGGTCAGAA CACAGGCGAC ACTTTCCTAA TTGCTTCTTT	720
GTTTTGGGCC GGAATCTTAA TATTCGAAGT GAATCTGATG CTGTGAGTTC TGATAGGAAT	780
TTCCCAAATT CAACAAATCT TCCAAGAAAT CCATCCATGG CAGATTATGA AGCACGGATC	840
TTTACTTTTG GGACATGGAT ATACTCAGTT AACAAGGAGC AGCTTGCAAG AGCTGGATTT	900
TATGCTTTAG GTGAAGGTGA TAAAGTAAAG TGCTTTCCTT GTGGAGGAGG GCTAACTGAT	960
TGGAAGCCCA GTGAAGACCC TTGGGAACAA CATGCTAAAT GGTATCCAGG GTGCAAATAT	1020
CTGTTAGAAC AGAAGGGACA AGAATATATA AACAAATATC ATTTAACTCA TTCACTTGAG	1080
GAGTGTCTGG TAAGAACTAC TGAGAAAACA CCATCACTAA CTAGAAGAAT TGATGATACC	1140
ATCTTCCAAA ATCCTATGGT ACAAGAAGCT ATACGAATGG GGTTCAGTTT CAAGGACATT	1200
AAGAAAATAA TGGAGGAAAA AATTCAGATA TCTGGGAGCA ACTATAAATC ACTTGAGGTT	1260
CTGGTTGCAG ATCTAGTGAA TGCTCAGAAA GACAGTATGC AAGATGAGTC AAGTCAGACT	1320
TCATTACAGA AAGAGATTAG TACTGAAGAG CAGCTAAGGC GCCTGCAAGA GGAGAAGCTT	1380

- 68 -

TGCAAAATCT GTATGGATAG AAATATTGCT ATCGTTTTTG TTCCTTGTGG ACATCTAGTC 1440
 ACTTGTAAC AATGTGCTGA AGCAGTTGAC AAGTGTCCTCA TGTGCTACAC AGTCATTACT 1500
 TTCAAGCAAA AAATTTTTAT GTCTTAATCT AACTCTATAG TAGGCATGTT ATGTTGTTCT 1560
 TATTACCCTG ATTGAATGTG TGATGTGAAC TGACTTTAAG TAATCAGGAT TGAATTCCAT 1620
 TAGCATTTGC TACCAAGTAG GAAAAAAAAT GTACATGGCA GTGTTTTAGT TGGCAATATA 1680
 ATCTTTGAAT TTCTTGATTT TTCAGGGTAT TAGCTGTATT ATCCATTTTT TTTACTGTTA 1740
 TTTAATTGAA ACCATAGACT AAGAATAAGA AGCATCATAC TATAACTGAA CACAATGTGT 1800
 ATTCATAGTA TACTGATTTA ATTTCTAAGT GTAAGTGAAT TAATCATCTG GATTTTTTAT 1860
 TCTTTTCAGA TAGGCTTAAC AAATGGAGCT TTCTGTATAT AAATGTGGAG ATTAGAGTTA 1920
 ATCTCCCCAA TCACATAATT TGTTTTGTGT GAAAAAGGAA TAAATTGTTC CATGCTGGTG 1980
 GAAAGATAGA GATTGTTTTT AGAGGTTGGT TGTGTGTTT TAGGATTCTG TCCATTTTCT 2040
 TGTAAGGGA TAAACACGGA CGTGTGCGAA ATATGTTTGT AAAGTGATTT GCCATTGTTG 2100
 AAAGCGTATT TAATGATAGA ATACTATCGA GCCAACATGT ACTGACATGG AAAGATGTCA 2160
 GAGATATGTT AAGTGTAATA TGCAAGTGGC GGGACACTAT GTATAGTCTG AGCCAGATCA 2220
 AAGTATGTAT GTTGTTAATA TGCATAGAAC GAGAGATTG GAAAGATATA CACCAAACTG 2280
 TTAAATGTGG TTTCTCTTCG GGGAGGGGGG GATTGGGGGA GGGGCCCCAG AGGGGTTTTA 2340
 GAGGGGCCTT TTCACTTTCG ACTTTTTTCA TTTTGTCTG TTCGGATTTT TTATAAGTAT 2400
 GTAGACCCCG AAGGGTTTTA TGGGAACATA CATCAGTAAC CTAACCCCG TGACTATCCT 2460
 GTGCTCTTCC TAGGGAGCTG TGTTGTTTCC CACCCACCAC CCTTCCCTCT GAACAAATGC 2520
 CTGAGTGCTG GGGCACTTTN 2540

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 497 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Thr Phe Asn Ser Phe Glu Gly Ser Lys Thr Cys Val Pro Ala Asp
 1 5 10 15
 Ile Asn Lys Glu Glu Glu Phe Val Glu Glu Phe Asn Arg Leu Lys Thr
 20 25 30
 Phe Ala Asn Phe Pro Ser Gly Ser Pro Val Ser Ala Ser Thr Leu Ala
 35 40 45

- 69 -

Arg Ala Gly Phe Leu Tyr Thr Gly Glu Gly Asp Thr Val Arg Cys Phe
 50 55 60
 Ser Cys His Ala Ala Val Asp Arg Trp Gln Tyr Gly Asp Ser Ala Val
 65 70 75 80
 Gly Arg His Arg Lys Val Ser Pro Asn Cys Arg Phe Ile Asn Gly Phe
 85 90 95
 Tyr Leu Glu Asn Ser Ala Thr Gln Ser Thr Asn Ser Gly Ile Gln Asn
 100 105 110
 Gly Gln Tyr Lys Val Glu Asn Tyr Leu Gly Ser Arg Asp His Phe Ala
 115 120 125
 Leu Asp Arg Pro Ser Glu Thr His Ala Asp Tyr Leu Leu Arg Thr Gly
 130 135 140
 Gln Val Val Asp Ile Ser Asp Thr Ile Tyr Pro Arg Asn Pro Ala Met
 145 150 155 160
 Tyr Cys Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr
 165 170 175
 Ala His Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr
 180 185 190
 Gly Ile Gly Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys
 195 200 205
 Asn Trp Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe
 210 215 220
 Pro Asn Cys Phe Phe Val Leu Gly Arg Asn Leu Asn Ile Arg Ser Glu
 225 230 235 240
 Ser Asp Ala Val Ser Ser Asp Arg Asn Phe Pro Asn Ser Thr Asn Leu
 245 250 255
 Pro Arg Asn Pro Ser Met Ala Asp Tyr Glu Ala Arg Ile Phe Thr Phe
 260 265 270
 Gly Thr Trp Ile Tyr Ser Val Asn Lys Glu Gln Leu Ala Arg Ala Gly
 275 280 285
 Phe Tyr Ala Leu Gly Glu Gly Asp Lys Val Lys Cys Phe His Cys Gly
 290 295 300
 Gly Gly Leu Thr Asp Trp Lys Pro Ser Glu Asp Pro Trp Glu Gln His
 305 310 315 320
 Ala Lys Trp Tyr Pro Gly Cys Lys Tyr Leu Leu Glu Gln Lys Gly Gln
 325 330 335
 Glu Tyr Ile Asn Asn Ile His Leu Thr His Ser Leu Glu Glu Cys Leu
 340 345 350
 Val Arg Thr Thr Glu Lys Thr Pro Ser Leu Thr Arg Arg Ile Asp Asp
 355 360 365
 Thr Ile Phe Gln Asn Pro Met Val Gln Glu Ala Ile Arg Met Gly Phe
 370 375 380

- 70 -

Ser Phe Lys Asp Ile Lys Lys Ile Met Glu Glu Lys Ile Gln Ile Ser
 385 390 395 400

Gly Ser Asn Tyr Lys S r Leu Glu Val Leu Val Ala Asp Leu Val Asn
 405 410 415

Ala Gln Lys Asp Ser Met Gln Asp Glu Ser Ser Gln Thr Ser Leu Gln
 420 425 430

Lys Glu Ile Ser Thr Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys
 435 440 445

Leu Cys Lys Ile Cys Met Asp Arg Asn Ile Ala Ile Val Phe Val Pro
 450 455 460

Cys Gly His Leu Val Thr Cys Lys Gln Cys Ala Glu Ala Val Asp Lys
 465 470 475 480

Cys Pro Met Cys Tyr Thr Val Ile Thr Phe Lys Gln Lys Ile Phe Met
 485 490 495

Ser

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2676 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCCTTGAGAT GTATCAGTAT AGGATTTAGG ATCTCCATGT TGGAACTCTA AATGCATAGA	60
AATGGAAATA ATGGAAATTT TTCATTTTGG CTTTTCAGCC TAGTATTAAA ACTGATAAAA	120
GCAAAGCCAT GCACAAAACT ACCTCCCTAG AGAAAGGCTA GTCCCTTTTC TTCCCCATTC	180
ATTCATTAT GAACATAGTA GAAAACAGCA TATTCTTATC AAATTGATG AAAAGCGCCA	240
ACACGTTTGA ACTGAAATAC GACTTGTCAT GTGAACTGTA CCGAATGTCT ACGTATTCCA	300
CTTTTCCTGC TGGGGTTCCT GTCTCAGAAA GGAGTCTTGC TCGTGCTGGT TTCTATTACA	360
CTGGTGTGAA TGACAAGGTC AAATGCTTCT GTTGTGGCCT GATGCTGGAT AACTGGAAAA	420
GAGGAGACAG TCCTACTGAA AAGCATAAAA AGTTGTATCC TAGCTGCAGA TTCGTTCAGA	480
GTCTAAATTC CGTTAACAAC TTGGAAGCTA CCTCTCAGCC TACTTTTCCT TCTTCAGTAA	540
CACATTCCAC AACTCATT A CTTCCGGGTA CAGAAAACAG TGGATATTTT CGTGGCTCTT	600
ATTCAAATTC TCCATCAAAT CCTGTAAACT CCAGAGCAAA TCAAGAATTT TCTGCCTTGA	660
TGAGAAGTTC CTACCCCTGT CCAATGAATA ACGAAAATGC CAGATTACTT ACTTTTCAGA	720

CATGGCCATT	GACTTTTCTG	TCGCCAACAG	ATCTGGCAGC	AGCAGGCTTT	TACTACATAG	780
GACCTGGAGA	CAGAGTGGCT	TGCTTTGCCT	GTGGTGGAAA	ATTGAGCAAT	TGGGAACCGA	840
AGGATAATGC	TATGTCAGAA	CACCTGAGAC	ATTTTCCCAA	ATGCCCATTT	ATAGAAAATC	900
AGCTTCAAGA	CACTTCAAGA	TACACAGTTT	CTAATCTGAG	CATGCAGACA	CATGCAGCCC	960
GCTTTAAAAAC	ATTCTTTAAC	TGGCCCTCTA	GTGTTCTAGT	TAATCCTGAG	CAGCTTGCAA	1020
GTGCGGGTTT	TTATTATGTG	GGTAACAGTG	ATGATGTCAA	ATGCTTTTGC	TGTGATGGTG	1080
GACTCAGGTG	TTGGGAATCT	GGAGATGATC	CATGGGTTCA	ACATGCCAAG	TGGTTTCCAA	1140
GGTGTGAGTA	CTTGATAAGA	ATTAAAGGAC	AGGAGTTCAT	CCGTCAAGTT	CAAGCCAGTT	1200
ACCCTCATCT	ACTTGAACAG	CTGCTATCCA	CATCAGACAG	CCCAGGAGAT	GAAAATGCAG	1260
AGTCATCAAT	TATCCATTG	GAACCTGGAG	AAGACCATTG	AGAAGATGCA	ATCATGATGA	1320
ATACTCCTGT	GATTAATGCT	GCCGTGGAAA	TGGGCTTTAG	TAGAAGCCTG	GTAAAACAGA	1380
CAGTTCAGAG	AAAAATCCTA	GCAACTGGAG	AGAATTATAG	ACTAGTCAAT	GATCTTGTGT	1440
TAGACTTACT	CAATGCAGAA	GATGAAATAA	GGGAAGAGGA	GAGAGAAAGA	GCAACTGAGG	1500
AAAAAGAATC	AAATGATTTA	TTATTAATCC	GGAAGAATAG	AATGGCACTT	TTTCAACATT	1560
TGACTTGTGT	AATTCCAATC	CTGGATAGTC	TACTAACTGC	CGGAATTATT	AATGAACAAG	1620
AACATGATGT	TATTAAACAG	AAGACACAGA	CGTCTTTACA	AGCAAGAGAA	CTGATTGATA	1680
CGATTTTAGT	AAAAGGAAAT	ATTGCAGCCA	CTGTATTGAG	AAACTCTCTG	CAAGAAGCTG	1740
AAGCTGTGTT	ATATGAGCAT	TTATTTGTGC	AACAGGACAT	AAAATATATT	CCCACAGAAG	1800
ATGTTTCAGA	TCTACCAAGT	GAAGAACAAT	TGCGGAGACT	ACCAGAAGAA	AGAACATGTA	1860
AAGTGTGTAT	GGACAAAGAA	GTGTCCATAG	TGTTTATTCC	TTGTGGTCAT	CTAGTAGTAT	1920
GCAAAGATTG	TGCTCCTTCT	TTAAGAAAGT	GTCTATTTTG	TAGGAGTACA	ATCAAGGGTA	1980
CAGTTCGTAC	ATTTCTTTCA	TGAAGAAGAA	CCAAAACATC	GTCTAAACTT	TAGAATTAAT	2040
TTATTAAATG	TATTATAACT	TTAACTTTTA	TCCTAATTTG	GTTTCCTTAA	AATTTTTTATT	2100
TATTTACAAC	TCAAAAAACA	TTGTTTTGTG	TAACATATTT	ATATATGTAT	CTAAACCATA	2160
TGAACATATA	TTTTTTAGAA	ACTAAGAGAA	TGATAGGCTT	TTGTTCTTAT	GAACGAAAAA	2220
GAGGTAGCAC	TACAAACACA	ATATTCAATC	CAAATTTTCA	CATTATTGAA	ATTGTAAGTG	2280
AAGTAAACT	TAAGATATTT	GAGTTAACCT	TTAAGAATTT	TAAATATTTT	GGCATTGTAC	2340
TAATACCGGG	AACATGAAGC	CAGGTGTGGT	GGTATGTACC	TGTAGTCCCA	GGCTGAGGCA	2400
AGAGAATTAC	TTGAGCCCAG	GAGTTTGAAT	CCATCCTGGG	CAGCATACTG	AGACCCTGCC	2460
TTTAAAAACN	AACAGNACCA	AANCCAAACA	CCAGGGACAC	ATTTCTCTGT	CTTTTTTGAT	2520
CAGTGTCCCTA	TACATCGAAG	GTGTGCATAT	ATGTTGAATC	ACATTTTAGG	GACATGGTGT	2580
TTTTATAAAG	AATTCTGTGA	GNAAAAATTT	AATAAAGCAA	CCAAATTACT	CTTAAAAAAA	2640

- 72 -

AAAAAAAAAA AAAAACTCG AGGGGCCCCGT ACCAAT

2676

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 604 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Met Asn Ile Val Glu Asn Ser Ile Phe Leu Ser Asn Leu Met Lys Ser
 1             5             10             15
Ala Asn Thr Phe Glu Leu Lys Tyr Asp Leu Ser Cys Glu Leu Tyr Arg
      20             25             30
Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro Val Ser Glu Arg
      35             40             45
Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys Val
      50             55             60
Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Arg Gly Asp
      65             70             75             80
Ser Pro Thr Glu Lys His Lys Lys Leu Tyr Pro Ser Cys Arg Phe Val
      85             90             95
Gln Ser Leu Asn Ser Val Asn Asn Leu Glu Ala Thr Ser Gln Pro Thr
      100            105            110
Phe Pro Ser Ser Val Thr His Ser Thr His Ser Leu Leu Pro Gly Thr
      115            120            125
Glu Asn Ser Gly Tyr Phe Arg Gly Ser Tyr Ser Asn Ser Pro Ser Asn
      130            135            140
Pro Val Asn Ser Arg Ala Asn Gln Glu Phe Ser Ala Leu Met Arg Ser
      145            150            155            160
Ser Tyr Pro Cys Pro Met Asn Asn Glu Asn Ala Arg Leu Leu Thr Phe
      165            170            175
Gln Thr Trp Pro Leu Thr Phe Leu Ser Pro Thr Asp Leu Ala Arg Ala
      180            185            190
Gly Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys
      195            200            205
Gly Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Asn Ala Met Ser Glu
      210            215            220
His Leu Arg His Phe Pro Lys Cys Pro Phe Ile Glu Asn Gln Leu Gln
      225            230            235            240

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- 73 -

Asp Thr Ser Arg Tyr Thr Val Ser Asn Leu Ser Met Gln Thr His Ala
 245 250 255
 Ala Arg Phe Lys Thr Phe Phe Asn Trp Pro Ser Ser Val Leu Val Asn
 260 265 270
 Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Asn Ser Asp
 275 280 285
 Asp Val Lys Cys Phe Cys Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser
 290 295 300
 Gly Asp Asp Pro Trp Val Gln His Ala Lys Trp Phe Pro Arg Cys Glu
 305 310 315 320
 Tyr Leu Ile Arg Ile Lys Gly Gln Glu Phe Ile Arg Gln Val Gln Ala
 325 330 335
 Ser Tyr Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Ser Pro
 340 345 350
 Gly Asp Glu Asn Ala Glu Ser Ser Ile Ile His Leu Glu Pro Gly Glu
 355 360 365
 Asp His Ser Glu Asp Ala Ile Met Met Asn Thr Pro Val Ile Asn Ala
 370 375 380
 Ala Val Glu Met Gly Phe Ser Arg Ser Leu Val Lys Gln Thr Val Gln
 385 390 395 400
 Arg Lys Ile Leu Ala Thr Gly Glu Asn Tyr Arg Leu Val Asn Asp Leu
 405 410 415
 Val Leu Asp Leu Leu Asn Ala Glu Asp Glu Ile Arg Glu Glu Glu Arg
 420 425 430
 Glu Arg Ala Thr Glu Glu Lys Glu Ser Asn Asp Leu Leu Leu Ile Arg
 435 440 445
 Lys Asn Arg Met Ala Leu Phe Gln His Leu Thr Cys Val Ile Pro Ile
 450 455 460
 Leu Asp Ser Leu Leu Thr Ala Gly Ile Ile Asn Glu Gln Glu His Asp
 465 470 475 480
 Val Ile Lys Gln Lys Thr Gln Thr Ser Leu Gln Ala Arg Glu Leu Ile
 485 490 495
 Asp Thr Ile Leu Val Lys Gly Asn Ile Ala Ala Thr Val Phe Arg Asn
 500 505 510
 Ser Leu Gln Glu Ala Glu Ala Val Leu Tyr Glu His Leu Phe Val Gln
 515 520 525
 Gln Asp Ile Lys Tyr Ile Pro Thr Glu Asp Val Ser Asp Leu Pro Val
 530 535 540
 Glu Glu Gln Leu Arg Arg Leu Pro Glu Glu Arg Thr Cys Lys Val Cys
 545 550 555 560
 Met Asp Lys Glu Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val
 565 570 575

- 74 -

Val Cys Lys Asp Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg
 580 585 590

Ser Thr Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
 595 600

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2580 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TTAGGTTACC TGAAAGAGTT ACTACAACCC CAAAGAGTTG TGTTCCTAAGT AGTATCTTGG	60
TAATTCAGAG AGATACTCAT CCTACCTGAA TATAAACTGA GATAAATCCA GTAAAGAAAG	120
TGTAGTAAAT TCTACATAAG AGTCTATCAT TGATTTCTTT TTGTGGTGGA AATCTTAGTT	180
CATGTGAAGA AATTCATGT GAATGTTTTA GCTATCAAAC AGTACTGTCA CCTACTCATG	240
CACAAACTG CCTCCCAAAG ACTTTTCCCA GGTCCCTCGT ATCAAAACAT TAAGAGTATA	300
ATGGAAGATA GCACGATCTT GTCAGATTGG ACAAACAGCA ACAAACAAA AATGAAGTAT	360
GACTTTTCCT GTGAACTCTA CAGAATGTCT ACATATTCAA CTTTCCCCGC CGGGGTGCCT	420
GTCTCAGAAA GGAGTCTTGC TCGTGCTGGT TTTTATTATA CTGGTGTGAA TGACAAGGTC	480
AAATGCTTCT GTTGTGGCCT GATGCTGGAT AACTGGAAAC TAGGAGACAG TCCTATTCAA	540
AAGCATAAAC AGCTATATCC TAGCTGTAGC TTTATTGAGA ATCTGGTTTC AGCTAGTCTG	600
GGATCCACCT CTAAGAATAC GTCTCCAATG AGAAACAGTT TTGCACATTC ATTATCTCCC	660
ACCTTGGAAC ATAGTAGCTT GTTCAGTGGT TCTTACTCCA GCCTTCCTCC AAACCCTCTT	720
AATTCTAGAG CAGTTGAAGA CATCTCTTCA TCGAGGACTA ACCCCTACAG TTATGCAATG	780
AGTACTGAAG AAGCCAGATT TCTTACCTAC CATATGTGGC CATTAACTTT TTTGTCACCA	840
TCAGAATTGG CAAGAGCTGG TTTTATTAT ATAGGACCTG GAGATAGGGT AGCCTGCTTT	900
GCCTGTGGTG GGAAGCTCAG TAACTGGGAA CCAAAGGATG ATGCTATGTC AGAACACCGG	960
AGGCATTTTC CCAACTGTCC ATTTTGGAA AATTCTCTAG AAACCTCTGAG GTTTAGCATT	1020
TCAAATCTGA GCATGCAGAC ACATGCAGCT CGAATGAGAA CATTTATGTA CTGGCCATCT	1080
AGTGTTCCAG TTCAGCCTGA GCAGCTGCA AGTGCTGGTT TTTATTATGT GGGTCGCAAT	1140
GATGATGTCA AATGCTTTGG TTGTGATGGT GGCTTGAGGT GTTGGGAATC TGGAGATGAT	1200
CCATGGGTAG AACATGCCAA GTGGTTTCCA AGGTGTGAGT TCTTGATACG AATGAAAGGC	1260

- 75 -

CAAGAGTTTG	TTGATGAGAT	TCAAGGTAGA	TATCCTCATC	TTCTTGAACA	GCTGTTGTCA	1320
ACTTCAGATA	CCACTGGAGA	AGAAAATGCT	GACCCACCAA	TTATTCATTT	TGGACCTGGA	1380
GAAAGTTCTT	CAGAAGATGC	TGTCATGATG	AATACACCTG	TGGTTAAATC	TGCCTTGGAA	1440
ATGGGCTTTA	ATAGAGACCT	GGTGAAACAA	ACAGTTCTAA	GTAAAATCCT	GACAACTGGA	1500
GAGAACTATA	AAACAGTTAA	TGATATTGTG	TCAGCACTTC	TTAATGCTGA	AGATGAAAAA	1560
AGAGAAGAGG	AGAAGGAAAA	ACAAGCTGAA	GAAATGGCAT	CAGATGATTT	GTCATTAATT	1620
CGGAAGAACA	GAATGGCTCT	CTTTCAACAA	TTGACATGTG	TGCTTCCTAT	CCTGGATAAT	1680
CTTTTAAAGG	CCAATGTAAT	TAATAAACAG	GAACATGATA	TTATTAAACA	AAAAACACAG	1740
ATACCTTTAC	AAGCGAGAGA	ACTGATTGAT	ACCATTGTTGG	TTAAAGGAAA	TGCTGCGGCC	1800
AACATCTTCA	AAAAGTGTCT	AAAAGAAATT	GACTCTACAT	TGTATAAGAA	CTTATTTGTG	1860
GATAAGAATA	TGAAGTATAT	TCCAACAGAA	GATGTTTCAG	GTCTGTCACT	GGAAGAACAA	1920
TTGAGGAGGT	TGCAAGAAGA	ACGAACTTGT	AAAGTGTGTA	TGGACAAAGA	AGTTTCTGTT	1980
GTATTTATTC	CTTGTTGGTCA	TCTGGTAGTA	TGCCAGGAAT	GTGCCCCTTC	TCTAAGAAAA	2040
TGCCCTATTT	GCAGGGGTAT	AATCAAGGGT	ACTGTTTCGTA	CATTTCTCTC	TTAAAGAAAA	2100
ATAGTCTATA	TTTTAACCTG	CATAAAAAGG	TCTTTAAAT	ATTGTTGAAC	ACTTGAAGCC	2160
ATCTAAAGTA	AAAAGGGAAT	TATGAGTTTT	TCAATTAGTA	ACATTCATGT	TCTAGTCTGC	2220
TTTGGTACTA	ATAATCTTGT	TTCTGAAAAG	ATGGTATCAT	ATATTTAATC	TTAATCTGTT	2280
TATTTACAAG	GGAAGATTTA	TGTTTGGTGA	ACTATATTAG	TATGTATGTG	TACCTAAGGG	2340
AGTAGCGTCN	CTGCTTGTTA	TGCATCATT	CAGGAGTTAC	TGGATTTGTT	GTTCTTTCAG	2400
AAAGCTTTGA	ANACTAAATT	ATAGTGTAGA	AAAGAACTGG	AAACCAGGAA	CTCTGGAGTT	2460
CATCAGAGTT	ATGGTGCCGA	ATTGTCTTTG	GTGCTTTTCA	CTTGTGTTTT	AAAATAAGGA	2520
TTTTTCTCTT	ATTTCTCCCC	CTAGTTTGTG	AGAAACATCT	CAATAAAGTG	CTTTAAAAAG	2580

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 618 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	His	Lys	Thr	Ala	Ser	Gln	Arg	Leu	Phe	Pro	Gly	Pro	Ser	Tyr	Gln
1				5					10					15	

- 76 -

Asn Ile Lys Ser Ile Met Glu Asp Ser Thr Ile Leu Ser Asp Trp Thr
 20 25 30
 Asn Ser Asn Lys Gln Lys Met Lys Tyr Asp Phe Ser Cys Glu Leu Tyr
 35 40 45
 Arg Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro Val Ser Glu
 50 55 60
 Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys
 65 70 75 80
 Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Leu Gly
 85 90 95
 Asp Ser Pro Ile Gln Lys His Lys Gln Leu Tyr Pro Ser Cys Ser Phe
 100 105 110
 Ile Gln Asn Leu Val Ser Ala Ser Leu Gly Ser Thr Ser Lys Asn Thr
 115 120 125
 Ser Pro Met Arg Asn Ser Phe Ala His Ser Leu Ser Pro Thr Leu Glu
 130 135 140
 His Ser Ser Leu Phe Ser Gly Ser Tyr Ser Ser Leu Pro Pro Asn Pro
 145 150 155 160
 Leu Asn Ser Arg Ala Val Glu Asp Ile Ser Ser Ser Arg Thr Asn Pro
 165 170 175
 Tyr Ser Tyr Ala Met Ser Thr Glu Glu Ala Arg Phe Leu Thr Tyr His
 180 185 190
 Met Trp Pro Leu Thr Phe Leu Ser Pro Ser Glu Leu Ala Arg Ala Gly
 195 200 205
 Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys Gly
 210 215 220
 Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Asp Ala Met Ser Glu His
 225 230 235 240
 Arg Arg His Phe Pro Asn Cys Pro Phe Leu Glu Asn Ser Leu Glu Thr
 245 250 255
 Leu Arg Phe Ser Ile Ser Asn Leu Ser Met Gln Thr His Ala Ala Arg
 260 265 270
 Met Arg Thr Phe Met Tyr Trp Pro Ser Ser Val Pro Val Gln Pro Glu
 275 280 285
 Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Arg Asn Asp Asp Val
 290 295 300
 Lys Cys Phe Gly Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser Gly Asp
 305 310 315 320
 Asp Pro Trp Val Glu His Ala Lys Trp Phe Pro Arg Cys Glu Phe Leu
 325 330 335
 Ile Arg Met Lys Gly Gln Glu Phe Val Asp Glu Ile Gln Gly Arg Tyr
 340 345 350

- 77 -

Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Thr Thr Gly Glu
 355 360 365
 Glu Asn Ala Asp Pro Pro Ile Ile His Phe Gly Pro Gly Glu Ser Ser
 370 375 380
 Ser Glu Asp Ala Val Met Met Asn Thr Pro Val Val Lys Ser Ala Leu
 385 390 395 400
 Glu Met Gly Phe Asn Arg Asp Leu Val Lys Gln Thr Val Leu Ser Lys
 405 410 415
 Ile Leu Thr Thr Gly Glu Asn Tyr Lys Thr Val Asn Asp Ile Val Ser
 420 425 430
 Ala Leu Leu Asn Ala Glu Asp Glu Lys Arg Glu Glu Glu Lys Glu Lys
 435 440 445
 Gln Ala Glu Glu Met Ala Ser Asp Asp Leu Ser Leu Ile Arg Lys Asn
 450 455 460
 Arg Met Ala Leu Phe Gln Gln Leu Thr Cys Val Leu Pro Ile Leu Asp
 465 470 475 480
 Asn Leu Leu Lys Ala Asn Val Ile Asn Lys Gln Glu His Asp Ile Ile
 485 490 495
 Lys Gln Lys Thr Gln Ile Pro Leu Gln Ala Arg Glu Leu Ile Asp Thr
 500 505 510
 Ile Trp Val Lys Gly Asn Ala Ala Ala Asn Ile Phe Lys Asn Cys Leu
 515 520 525
 Lys Glu Ile Asp Ser Thr Leu Tyr Lys Asn Leu Phe Val Asp Lys Asn
 530 535 540
 Met Lys Tyr Ile Pro Thr Glu Asp Val Ser Gly Leu Ser Leu Glu Glu
 545 550 555 560
 Gln Leu Arg Arg Leu Gln Glu Glu Arg Thr Cys Lys Val Cys Met Asp
 565 570 575
 Lys Glu Val Ser Val Val Phe Ile Pro Cys Gly His Leu Val Val Cys
 580 585 590
 Gln Glu Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg Gly Ile
 595 600 605
 Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
 610 615

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2100 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

- 78 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACACTCTGC	TGGGCGGCGG	GCCGCCCTCC	TCCGGGACCT	CCCCTCGGGA	ACCGTCGCCC	60
GCGGCGCTTA	GTTAGGACTG	GAGTGCTTGG	CGCGAAAAGG	TGGACAAGTC	CTATTTTCCA	120
GAGAAGATGA	CTTTTAACAG	TTTGAAGGA	ACTAGAACTT	TTGTACTTGC	AGACACCAAT	180
AAGGATGAAG	AATTTGTAGA	AGAGTTTAAT	AGATTAAAAA	CATTTGCTAA	CTTCCCAAGT	240
AGTAGTCCTG	TTTCAGCATC	AACATTGGCG	CGAGCTGGGT	TTCTTTATAC	CGGTGAAGGA	300
GACACCGTGC	AATGTTTCAG	TTGTCATGCG	GCAATAGATA	GATGGCAGTA	TGGAGACTCA	360
GCTGTTGGAA	GACACAGGAG	AATATCCCCA	AATTGCAGAT	TTATCAATGG	TTTTTATTTT	420
GAAATGGTG	CTGCACAGTC	TACAAATCCT	GGTATCCAAA	ATGGCCAGTA	CAAATCTGAA	480
AACTGTGTGG	GAAATAGAAA	TCCTTTTGCC	CCTGACAGGC	CACCTGAGAC	TCATGCTGAT	540
TATCTCTTGA	GAACGGACA	GGTTGTAGAT	ATTCAGACA	CCATATACCC	GAGGAACCCT	600
GCCATGTGTA	GTGAAGAAGC	CAGATTGAAG	TCATTTCAGA	ACTGGCCGGA	CTATGCTCAT	660
TTAACCCCCA	GAGAGTTAGC	TAGTGCTGGC	CTCTACTACA	CAGGGGCTGA	TGATCAAGTG	720
CAATGCTTTT	GTTGTGGGGG	AAAAC TGAAA	AATTGGGAAC	CCTGTGATCG	TGCCTGGTCA	780
GAACACAGGA	GACACTTTCC	CAATTGCTTT	TTTGTTTTGG	GCCGGAACGT	TAATGTTCGA	840
AGTGAATCTG	GTGTGAGTTC	TGATAGGAAT	TTCCCAAATT	CAACAAACTC	TCCAAGAAAT	900
CCAGCCATGG	CAGAATATGA	AGCACGGATC	GTTACTTTTG	GAACATGGAT	ATACTCAGTT	960
AACAAGGAGC	AGCTTGCAAG	AGCTGGATTT	TATGCTTTAG	GTGAAGGCGA	TAAAGTGAAG	1020
TGCTTCCACT	GTGGAGGAGG	GCTCACGGAT	TGGAAGCCAA	GTGAAGACCC	CTGGGACCAG	1080
CATGCTAAGT	GCTACCCAGG	GTGCAAATAC	CTATTGGATG	AGAAGGGGCA	AGAATATATA	1140
ATAATATTC	ATTTAACCCA	TCCACTTGAG	GAATCTTTGG	GAAGAACTGC	TGAAAAAACA	1200
CCACCGCTAA	CTAAAAAAT	CGATGATACC	ATCTTCAGA	ATCCTATGGT	GCAAGAAGCT	1260
ATACGAATGG	GATTTAGCTT	CAAGGACCTT	AAGAAAACAA	TGGAAGAAAA	AATCCAAACA	1320
TCCGGGAGCA	GCTATCTATC	ACTTGAGGTC	CTGATTGCAG	ATCTTGTGAG	TGCTCAGAAA	1380
GATAATACGG	AGGATGAGTC	AAGTCAAACT	TCATTGCAGA	AAGACATTAG	TACTGAAGAG	1440
CAGCTAAGGC	GCCTACAAGA	GGAGAAGCTT	TCCAAAATCT	GTATGGATAG	AAATATTGCT	1500
ATCGTTTTTT	TTCTTGTTGG	ACATCTGGCC	ACTTGTAAC	AGTGTGCAGA	AGCAGTTGAC	1560
AAATGTCCCA	TGTGCTACAC	CGTCATTACG	TTCAACCAAA	AAATTTTTAT	GTCTTAGTGG	1620
GGCACCACAT	GTTATGTTCT	TCTTGCTCTA	ATTGAATGTG	TAATGGGAGC	GAACTTTAAG	1680
TAATCCTGCA	TTTGCAATCC	ATTAGCATCC	TGCTGTTTCC	AAATGGAGAC	CAATGCTAAC	1740
AGCACTGTTT	CCGTCTAAAC	ATTCAATTC	TGGATCTTTC	GAGTTATCAG	CTGTATCATT	1800
TAGCCAGTGT	TTTACTCGAT	TGAAACCTTA	GACAGAGAAG	CATTTTATAG	CTTTTCACAT	1860

- 79 -

GTATATTGGT AGTACACTGA CTTGATTTCT ATATGTAAGT GAATTCATCA CCTGCATGTT 1920
 TCATGCCTTT TGCATAAGCT TAACAAATGG AGTGTTCTGT ATAAGCATGG AGATGTGATG 1980
 GAATCTGCCC AATGACTTTA ATTGGCTTAT TGTAACACAG GAAAGAACTG CCCCACGCTG 2040
 CTGGGAGGAT AAAGATTGTT TTAGATGCTC ACTTCTGTGT TTTAGGATTC TGCCCATTTA 2100

(2). INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 496 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met	Thr	Phe	Asn	Ser	Phe	Glu	Gly	Thr	Arg	Thr	Phe	Val	Leu	Ala	Asp	1	5	10	15
Thr	Asn	Lys	Asp	Glu	Glu	Phe	Val	Glu	Glu	Phe	Asn	Arg	Leu	Lys	Thr	20	25	30	
Phe	Ala	Asn	Phe	Pro	Ser	Ser	Ser	Pro	Val	Ser	Ala	Ser	Thr	Leu	Ala	35	40	45	
Arg	Ala	Gly	Phe	Leu	Tyr	Thr	Gly	Glu	Gly	Asp	Thr	Val	Gln	Cys	Phe	50	55	60	
Ser	Cys	His	Ala	Ala	Ile	Asp	Arg	Trp	Gln	Tyr	Gly	Asp	Ser	Ala	Val	65	70	75	80
Gly	Arg	His	Arg	Arg	Ile	Ser	Pro	Asn	Cys	Arg	Phe	Ile	Asn	Gly	Phe	85	90	95	
Tyr	Phe	Glu	Asn	Gly	Ala	Ala	Gln	Ser	Thr	Asn	Pro	Gly	Ile	Gln	Asn	100	105	110	
Gly	Gln	Tyr	Lys	Ser	Glu	Asn	Cys	Val	Gly	Asn	Arg	Asn	Pro	Phe	Ala	115	120	125	
Pro	Asp	Arg	Pro	Pro	Glu	Thr	His	Ala	Asp	Tyr	Leu	Leu	Arg	Thr	Gly	130	135	140	
Gln	Val	Val	Asp	Ile	Ser	Asp	Thr	Ile	Tyr	Pro	Arg	Asn	Pro	Ala	Met	145	150	155	160

- 80 -

Cys Ser Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr
 165 170 175
 Ala His Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr
 180 185 190
 Gly Ala Asp Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys
 195 200 205
 Asn Trp Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe
 210 215 220
 Pro Asn Cys Phe Phe Val Leu Gly Arg Asn Val Asn Val Arg Ser Glu
 225 230 235 240
 Ser Gly Val Ser Ser Asp Arg Asn Phe Pro Asn Ser Thr Asn Ser Pro
 245 250 255
 Arg Asn Pro Ala Met Ala Glu Tyr Glu Ala Arg Ile Val Thr Phe Gly
 260 265 270
 Thr Trp Ile Tyr Ser Val Asn Lys Glu Gln Leu Ala Arg Ala Gly Phe
 275 280 285
 Tyr Ala Leu Gly Glu Gly Asp Lys Val Lys Cys Phe His Cys Gly Gly
 290 295 300
 Gly Leu Thr Asp Trp Lys Pro Ser Glu Asp Pro Trp Asp Gln His Ala
 305 310 315 320
 Lys Cys Tyr Pro Gly Cys Lys Tyr Leu Leu Asp Glu Lys Gly Gln Glu
 325 330 335
 Tyr Ile Asn Asn Ile His Leu Thr His Pro Leu Glu Glu Ser Leu Gly
 340 345 350
 Arg Thr Ala Glu Lys Thr Pro Pro Leu Thr Lys Lys Ile Asp Asp Thr
 355 360 365
 Ile Phe Gln Asn Pro Met Val Gln Glu Ala Ile Arg Met Gly Phe Ser
 370 375 380
 Phe Lys Asp Leu Lys Lys Thr Met Glu Glu Lys Ile Gln Thr Ser Gly
 385 390 395 400
 Ser Ser Tyr Leu Ser Leu Glu Val Leu Ile Ala Asp Leu Val Ser Ala
 405 410 415
 Gln Lys Asp Asn Thr Glu Asp Glu Ser Ser Gln Thr Ser Leu Gln Lys
 420 425 430
 Asp Ile Ser Thr Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys Leu
 435 440 445
 Ser Lys Ile Cys Met Asp Arg Asn Ile Ala Ile Val Phe Phe Pro Cys
 450 455 460
 Gly His Leu Ala Thr Cys Lys Gln Cys Ala Glu Ala Val Asp Lys Cys
 465 470 475 480
 Pro Met Cys Tyr Thr Val Ile Thr Phe Asn Gln Lys Ile Phe Met Ser
 485 490 495

- 81 -

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 67 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```

Lys Ala Ala Arg Leu Gly Thr Tyr Thr Asn Trp Pro Val Gln Phe Leu
1           5           10           15
Glu Pro Ser Arg Met Ala Ala Ser Gly Phe Tyr Tyr Leu Gly Arg Gly
20           25
Asp Glu Val Arg Cys Ala Phe Cys Lys Val Glu Ile Thr Asn Trp Val
35           40           45
Arg Gly Asp Asp Pro Glu Thr Asp His Lys Arg Trp Ala Pro Gln Cys
50           55           60
Pro Phe Val
65

```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 275 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Met Ser Asp Leu Arg Leu Glu Glu Val Arg Leu Asn Thr Phe Glu Lys
1           5           10           15
Trp Pro Val Ser Phe Leu Ser Pro Glu Thr Met Ala Lys Asn Gly Phe
20           25
Tyr Tyr Leu Gly Arg Ser Asp Glu Val Arg Cys Ala Phe Cys Lys Val
35           40           45
Glu Ile Met Arg Trp Lys Glu Gly Glu Asp Pro Ala Ala Asp His Lys
50           55           60
Lys Trp Ala Pro Gln Cys Pro Phe Val Lys Gly Ile Asp Val Cys Gly
65           70           75           80
Ser Ile Val Thr Thr Asn Asn Ile Gln Asn Thr Thr Thr His Asp Thr
85           90           95

```

- 82 -

```

11  Ile Gly Pro Ala His Pro Lys Tyr Ala His Glu Ala Ala Arg Val
      100              105              110

Lys Ser Phe His Asn Trp Pro Arg Cys Met Lys Gln Arg Pro Glu Gln
      115              120              125

Met Ala Asp Ala Gly Phe Phe Tyr Thr Gly Tyr Gly Asp Asn Thr Lys
      130              135              140

Cys Phe Tyr Cys Asp Gly Leu Lys Asp Trp Glu Pro Glu Asp Val
      145              150              155              160

Pro Trp Glu Gln His Val Arg Trp Phe Asp Arg Cys Ala Tyr Val Gln
      165              170              175

Leu Val Lys Gly Arg Asp Tyr Val Gln Lys Val Ile Thr Glu Ala Cys
      180              185              190

Val Leu Pro Gly Glu Asn Thr Thr Val Ser Thr Ala Ala Pro Val Ser
      195              200              205

Glu Pro Ile Pro Glu Thr Lys Ile Glu Lys Glu Pro Gln Val Glu Asp
      210              215              220

Ser Lys Leu Cys Lys Ile Cys Tyr Val Glu Glu Cys Ile Val Cys Phe
      225              230              235              240

Val Pro Cys Gly His Val Val Ala Cys Ala Lys Cys Ala Leu Ser Val
      245              250              255

Asp Lys Cys Pro Met Cys Arg Lys Ile Val Thr Ser Val Leu Lys Val
      260              265              270

Tyr Phe Ser
      275

```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 498 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```

Met Thr Glu Leu Gly Met Glu Leu Glu Ser Val Arg Leu Ala Thr Phe
1      5      10

Gly Glu Trp Pro Leu Asn Ala Pro Val Ser Ala Glu Asp Leu Val Ala
      20      25      30

Asn Gly Phe Phe Ala Thr Gly Lys Trp Leu Glu Ala Glu Cys His Phe
      35      40      45

Cys His Val Arg Ile Asp Arg Trp Glu Tyr Gly Asp Gln Val Ala Glu
      50      55      60

```

- 83 -

Arg His Arg Arg Ser Ser Pro Ile Cys Ser Met Val Leu Ala Pro Asn
 65 70 75 80
 His Cys Gly Asn Val Pro Arg Ser Gln Glu Ser Asp Asn Glu Gly Asn
 85 90 95
 Ser Val Val Asp Ser Pro Glu Ser Cys Ser Cys Pro Asp Leu Leu Leu
 100 105 110
 Glu Ala Asn Arg Leu Val Thr Phe Lys Asp Trp Pro Asn Pro Asn Ile
 115 120 125
 Thr Pro Gln Ala Leu Ala Lys Ala Gly Phe Tyr Tyr Leu Asn Arg Leu
 130 135 140
 Asp His Val Lys Cys Val Trp Cys Asn Gly Val Ile Ala Lys Trp Glu
 145 150 155 160
 Lys Asn Asp Asn Ala Phe Glu Glu His Lys Arg Phe Phe Pro Gln Cys
 165 170 175
 Pro Arg Val Gln Met Gly Pro Leu Ile Glu Phe Ala Thr Gly Lys Asn
 180 185 190
 Leu Asp Glu Leu Gly Ile Gln Pro Thr Thr Leu Pro Leu Arg Pro Lys
 195 200 205
 Tyr Ala Cys Val Asp Ala Arg Leu Arg Thr Phe Thr Asp Trp Pro Ile
 210 215 220
 Ser Asn Ile Gln Pro Ala Ser Ala Leu Ala Gln Ala Gly Leu Tyr Tyr
 225 230 235 240
 Gln Lys Ile Gly Asp Gln Val Arg Cys Phe His Cys Asn Ile Gly Leu
 245 250 255
 Arg Ser Trp Gln Lys Glu Asp Glu Pro Trp Phe Glu His Ala Lys Trp
 260 265 270
 Ser Pro Lys Cys Gln Phe Val Leu Leu Ala Lys Gly Pro Ala Tyr Val
 275 280 285
 Ser Glu Val Leu Ala Thr Thr Ala Ala Asn Ala Ser Ser Gln Pro Ala
 290 295 300
 Thr Ala Pro Ala Pro Thr Leu Gln Ala Asp Val Leu Met Asp Glu Ala
 305 310 315 320
 Pro Ala Lys Glu Ala Leu Thr Leu Gly Ile Asp Gly Gly Val Val Arg
 325 330 335
 Asn Ala Ile Gln Arg Lys Leu Leu Ser Ser Gly Cys Ala Phe Ser Thr
 340 345 350
 Leu Asp Glu Leu Leu His Asp Ile Phe Asp Asp Ala Gly Ala Gly Ala
 355 360 365
 Ala Leu Glu Val Arg Glu Pro Pro Glu Pro Ser Ala Pro Phe Ile Glu
 370 375 380
 Pro Cys Gln Ala Thr Thr Ser Lys Ala Ala Ser Val Pro Ile Pro Val
 385 390 395 400

- 84 -

Ala Asp Ser Ile Pro Ala Lys Pro Gln Ala Ala Glu Ala Val Ser Asn
 405 410 415

Ile Ser Lys Ile Thr Asp Glu Ile Gln Lys Met Ser Val Ser Thr Pro
 420 425 430

Asn Gly Asn Leu Ser Leu Glu Glu Glu Asn Arg Gln Leu Lys Asp Ala
 435 440 445

Arg Leu Cys Lys Val Cys Leu Asp Glu Glu Val Gly Val Val Phe Leu
 450 455 460

Pro Cys Gly His Leu Ala Thr Cys Asn Gln Cys Ala Pro Ser Val Ala
 465 470 475 480

Asn Cys Pro Met Cys Arg Ala Asp Ile Lys Gly Phe Val Arg Thr Phe
 485 490 495

Leu Ser

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 67 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Glu Glu Val Arg Leu Asn Thr Phe Glu Lys Trp Pro Val Ser Phe Leu
 1 5 10 15

Ser Pro Glu Thr Met Ala Lys Asn Gly Phe Tyr Tyr Leu Gly Arg Ser
 20 25 30

Asp Glu Val Arg Cys Ala Phe Cys Lys Val Glu Ile Met Arg Trp Lys
 35 40 45

Glu Gly Glu Asp Pro Ala Ala Asp His Lys Lys Trp Ala Pro Gln Cys
 50 55 60

Pro Phe Val
 65

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 67 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

(2) INFORMATION FOR SEQ ID NO:16:

(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

(2) INFORMATION FOR SEQ ID NO:17:

(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

- 86 -

Glu Phe Asn Arg Leu Lys Thr Phe Ala Asn Phe Pro Ser Gly Ser Pro
 1 5 10 15
 Val Ser Ala Ser Thr Leu Ala Arg Ala Gly Phe Leu Tyr Thr Gly Glu
 20 25 30
 Gly Asp Thr Val Arg Cys Phe Ser Cys His Ala Ala Val Asp Arg Trp
 35 40 45
 Gln Tyr Gly Asp Ser Ala Val Gly Arg His Arg Lys Val Ser Pro Asn
 50 55 60
 Cys Arg Phe Ile
 65

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 68 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant
 - (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Glu Leu Tyr Arg Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro
 1 5 10 15
 Val Ser Glu Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val
 20 25 30
 Asn Asp Lys Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp
 35 40 45
 Lys Arg Gly Asp Ser Pro Thr Glu Lys His Lys Lys Leu Tyr Pro Ser
 50 55 60
 Cys Arg Phe Val
 65

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 68 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant
 - (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Glu Leu Tyr Arg Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro
 1 5 10 15

- 87 -

Val Ser Glu Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val
 20 25 30

Asn Asp Lys Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp
 35 40 45

Lys Leu Gly Asp Ser Pro Ile Gln Lys His Lys Gln Leu Tyr Pro Ser
 50 55 60

Cys Ser Phe Ile
 65

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 68 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant
 - (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr Ala His
 1 5 10 15

Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr Gly Ala
 20 25 30

Asp Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys Asn Trp
 35 40 45

Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe Pro Asn
 50 55 60

Cys Phe Phe Val
 65

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 68 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant
 - (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr Ala His
 1 5 10 15

Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr Gly Ile
 20 25 30

- 88 -

Gly Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys L u Lys Asn Trp
 35 40 45

Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe Pro Asn
 50 55 60

Cys Phe Phe Val
 65

(2). INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 67 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Glu Asn Ala Arg Leu Leu Thr Phe Gln Thr Trp Pro Leu Thr Phe Leu
 1 5 10 15

Ser Pro Thr Asp Leu Ala Arg Ala Gly Phe Tyr Tyr Ile Gly Pro Gly
 20 25 30

Asp Arg Val Ala Cys Phe Ala Cys Gly Gly Lys Leu Ser Asn Trp Glu
 35 40 45

Pro Lys Asp Asn Ala Met Ser Glu His Leu Arg His Phe Pro Lys Cys
 50 55 60

Pro Phe Ile
 65

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 67 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Glu Glu Ala Arg Phe Leu Thr Tyr His Met Trp Pro Leu Thr Phe Leu
 1 5 10 15

Ser Pro Ser Glu Leu Ala Arg Ala Gly Phe Tyr Tyr Ile Gly Pro Gly
 20 25 30

Asp Arg Val Ala Cys Phe Ala Cys Gly Gly Lys Leu Ser Asn Trp Glu
 35 40 45

- 89 -

Pro Lys Asp Asp Ala Met Ser Glu His Arg Arg His Phe Pro Asn Cys
 50 55 60

Pro Phe Leu
 65

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 66 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Tyr Glu Ala Arg Ile Val Thr Phe Gly Thr Trp Ile Tyr Ser Val Asn
 1 5 10 15
 Lys Glu Gln Leu Ala Arg Ala Gly Phe Tyr Ala Leu Gly Glu Gly Asp
 20 25 30
 Lys Val Lys Cys Phe His Cys Gly Gly Gly Leu Thr Asp Trp Lys Pro
 35 40 45
 Ser Glu Asp Pro Trp Asp Gln His Ala Lys Cys Tyr Pro Gly Cys Lys
 50 55 60
 Tyr Leu
 65

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 66 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Tyr Glu Ala Arg Ile Phe Thr Phe Gly Thr Trp Ile Tyr Ser Val Asn
 1 5 10 15
 Lys Glu Gln Leu Ala Arg Ala Gly Phe Tyr Ala Leu Gly Glu Gly Asp
 20 25 30
 Lys Val Lys Cys Phe His Cys Gly Gly Gly Leu Thr Asp Trp Lys Pro
 35 40 45
 Ser Glu Asp Pro Trp Glu Gln His Ala Lys Trp Tyr Pro Gly Cys Lys
 50 55 60

- 90 -

Tyr Leu
65

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```

His Ala Ala Arg Phe Lys Thr Phe Phe Asn Trp Pro Ser Ser Val Leu
1           5           10           15
Val Asn Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Asn
20           25           30
Ser Asp Asp Val Lys Cys Phe Cys Cys Asp Gly Gly Leu Arg Cys Trp
35           40           45
Glu Ser Gly Asp Asp Pro Trp Val Gln His Ala Lys Trp Phe Pro Arg
50           55           60
Cys Glu Tyr Leu
65

```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```

His Ala Ala Arg Met Arg Thr Phe Met Tyr Trp Pro Ser Ser Val Pro
1           5           10           15
Val Gln Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Arg
20           25           30
Asn Asp Asp Val Lys Cys Phe Gly Cys Asp Gly Gly Leu Arg Cys Trp
35           40           45
Glu Ser Gly Asp Asp Pro Trp Val Glu His Ala Lys Trp Phe Pro Arg
50           55           60
Cys Glu Phe Leu
65

```

- 91 -

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 68 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Glu Ala Ala Arg Leu Arg Thr Phe Ala Glu Trp Pro Arg Gly Leu Lys
 1 5 10 15
 Gln Arg Pro Glu Glu Leu Ala Glu Ala Gly Phe Phe Tyr Thr Gly Gln
 20 25 30
 Gly Asp Lys Thr Arg Cys Phe Cys Cys Asp Gly Gly Leu Lys Asp Trp
 35 40 45
 Glu Pro Asp Asp Ala Pro Trp Gln Gln His Ala Arg Trp Tyr Asp Arg
 50 55 60
 Cys Glu Tyr Val
 65

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 68 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Glu Ala Ala Arg Val Lys Ser Phe His Asn Trp Pro Arg Cys Met Lys
 1 5 10 15
 Gln Arg Pro Glu Gln Met Ala Asp Ala Gly Phe Phe Tyr Thr Gly Tyr
 20 25 30
 Gly Asp Asn Thr Lys Cys Phe Tyr Cys Asp Gly Gly Leu Lys Asp Trp
 35 40 45
 Glu Pro Glu Asp Val Pro Trp Glu Gln His Val Arg Trp Phe Asp Arg
 50 55 60
 Cys Ala Tyr Val
 65

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:

- 92 -

- (A) LENGTH: 68 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```

Val Asp Ala Arg Leu Arg Thr Phe Thr Asp Trp Pro Ile Ser Asn Ile
1           5           10           15
Gln Pro Ala Ser Ala Leu Ala Gln Ala Gly Leu Tyr Tyr Gln Lys Ile
          20           25           30
Gly Asp Gln Val Arg Cys Phe His Cys Asn Ile Gly Leu Arg Ser Trp
          35           40           45
Gln Lys Glu Asp Glu Pro Trp Phe Glu His Ala Lys Trp Ser Pro Lys
          50           55           60
Cys Gln Phe Val
          65

```

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 66 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant
 - (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```

Glu Ser Val Arg Leu Ala Thr Phe Gly Glu Trp Pro Leu Asn Ala Pro
1           5           10           15
Val Ser Ala Glu Asp Leu Val Ala Asn Gly Phe Phe Gly Thr Trp Met
          20           25           30
Glu Ala Glu Cys Asp Phe Cys His Val Arg Ile Asp Arg Trp Glu Tyr
          35           40           45
Gly Asp Leu Val Ala Glu Arg His Arg Arg Ser Ser Pro Ile Cys Ser
          50           55           60
Met Val
          65

```

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 46 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant

- 93 -

(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Glu	Gln	Leu	Arg	Arg	Leu	Gln	Glu	Glu	Arg	Thr	Cys	Lys	Val	Cys	Met
1				5					10					15	
Asp	Lys	Glu	Val	Ser	Val	Val	Phe	Ile	Pro	Cys	Gly	His	Leu	Val	Val
		20						25					30		
Cys	Gln	Glu	Cys	Ala	Pro	Ser	Leu	Arg	Lys	Cys	Pro	Ile	Cys		
		35					40					45			

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Glu	Gln	Leu	Arg	Arg	Leu	Pro	Glu	Glu	Arg	Thr	Cys	Lys	Val	Cys	Met
1				5					10					15	
Asp	Lys	Glu	Val	Ser	Ile	Val	Phe	Ile	Pro	Cys	Gly	His	Leu	Val	Val
		20						25					30		
Cys	Lys	Asp	Cys	Ala	Pro	Ser	Leu	Arg	Lys	Cys	Pro	Ile	Cys		
		35					40					45			

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Glu	Gln	Leu	Arg	Arg	Leu	Gln	Glu	Glu	Lys	Leu	Ser	Lys	Ile	Cys	Met
1				5					10					15	
Asp	Arg	Asn	Ile	Ala	Ile	Val	Phe	Phe	Pro	Cys	Gly	His	Leu	Ala	Thr
		20						25					30		

- 94 -

Cys Lys Gln Cys Ala Glu Ala Val Asp Lys Cys Pro Met Cys
 35 40 45

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys Leu Cys Lys Ile Cys Met
 1 5 10 15
 Asp Arg Asn Ile Ala Ile Val Phe Val Pro Cys Gly His Leu Val Thr
 20 25 30
 Cys Lys Gln Cys Ala Glu Ala Val Asp Lys Cys Pro Met Cys
 35 40 45

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Glu Glu Asn Arg Gln Leu Lys Asp Ala Arg Leu Cys Lys Val Cys Leu
 1 5 10 15
 Asp Glu Glu Val Gly Val Val Phe Leu Pro Cys Gly His Leu Ala Thr
 20 25 30
 Cys Asn Gln Cys Ala Pro Ser Val Ala Asn Cys Pro Met Cys
 35 40 45

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

- 95 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Glu Lys Glu Pro Gln Val Glu Asp Ser Lys Leu Cys Lys Ile Cys Tyr
 1 5 10 15
 Val Glu Glu Cys Ile Val Cys Phe Val Pro Cys Gly His Val Val Ala
 20 25 30
 Cys Ala Lys Cys Ala Leu Ser Val Asp Lys Cys Pro Met Cys
 35 40 45

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Ala Val Glu Ala Glu Val Ala Asp Asp Arg Leu Cys Lys Ile Cys Leu
 1 5 10 15
 Gly Ala Glu Lys Thr Val Cys Phe Val Pro Cys Gly His Val Val Ala
 20 25 30
 Cys Gly Lys Cys Ala Ala Gly Val Thr Thr Cys Pro Val Cys
 35 40 45

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2474 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GAATTCCGGG AGACCTACAC CCCC GGAGAT CAGAGGTCAT TGCTGGCGTT CAGAGCCTAG 60
 GAAGTGGGCT GCGGTATCAG CCTAGCAGTA AAACCGACCA GAAGCCATGC 120
 ATCCCCAGAG AAAGACTTGT CCCTTCCCCT CCCTGTCATC TCACCATGAA CATGGTTCAA 180
 GACAGCGCCT TTCTAGCCAA GCTGATGAAG AGTGCTGACA CCTTTGAGTT GAAGTATGAC 240
 TTTTCCTGTG AGCTGTACCG ATTGTCCACG TATTCAGCTT TTCCCAGGGG AGTTCCTGTG 300
 TCAGAAAGGA GTCTGGCTCG TGCTGGCTTT TACTACACTG GTGCCAATGA CAAGGTCAAG 360
 TGCTTCTGCT GTGGCCTGAT GCTAGACAAC TGGAAACAAG GGGACAGTCC CATGGAGAAG 420

- 96 -

CACAGAAAGT	TGTACCCAG	CTGCAACTTT	GTACAGACTT	TGAATCCAGC	CAACAGTCTG	480
GAAGCTAGTC	CTCGGCCTTC	TCTTCCTTCC	ACGGCGATGA	GCACCATGCC	TTTGAGCTTT	540
GCAAGTTCTG	AGAATACTGG	CTATTTTCAGT	GGCTCTTACT	CGAGCTTTCC	CTCAGACCCT	600
GTGAACTTCC	GAGCAAATCA	AGATTGTCCT	GCTTTGAGCA	CAAGTCCCTA	CCACTTTGCA	660
ATGAACACAG	AGAAGGCCAG	ATTACTCACC	TATGAAACAT	GGCCATTGTC	TTTTCTGTCA	720
CCAGCAAAGC	TGGCCAAAGC	AGGCTTCTAC	TACATAGGAC	CTGGAGATAG	AGTGGCCTGC	780
TTTGCGTGCG	ATGGGAAACT	GAGCAACTGG	GAACGTAAGG	ATGATGCTAT	GTCAGAGCAC	840
CAGAGGCATT	TCCCCAGCTG	TCCGTTCTTA	AAAGACTTGG	GTCAGTCTGC	TTCGAGATAC	900
ACTGTCTCTA	ACCTGAGCAT	GCAGACACAC	GCAGCCCGTA	TTAGAACATT	CTCTAACTGG	960
CCTTCTAGTG	CACTAGTTCA	TTCCCAGGAA	CTTGCAAGTG	CGGGCTTTTA	TTATACAGGA	1020
CACAGTGATG	ATGTCAAGTG	TTTATGCTGT	GATGGTGGGC	TGAGGTGCTG	GGAATCTGGA	1080
GATGACCCCT	GGGTGGAACA	TGCCAAGTGG	TTTCCAAGGT	GTGAGTACTT	GCTCAGAATC	1140
AAAGGCCAAG	AATTTGTCAG	CCAAGTTCAA	GCTGGCTATC	CTCATCTACT	TGAGCAGCTA	1200
TTATCTACGT	CAGACTCCCC	AGAAGATGAG	AATGCAGACG	CAGCAATCGT	GCATTTTGGC	1260
CCTGGAGAAA	GTTCGGAAGA	TGTCGTCATG	ATGAGCACGC	CTGTGCTTAA	AGCAGCCTTG	1320
GAAATGGGCT	TCAGTAGGAG	CCTGGTGAGA	CAGACGGTTC	AGTGGCAGAT	CCTGGCCACT	1380
GGTGAGAACT	ACAGGACCGT	CAGTGACCTC	GTTATAGGCT	TACTCGATGC	AGAAGACGAG	1440
ATGAGAGAGG	AGCAGATGGA	GCAGGCGGCC	GAGGAGGAGG	AGTCAGATGA	TCTAGCACTA	1500
ATCCGGAAGA	ACAAAATGGT	GCTTTTCCAA	CATTTGACGT	GTGTGACACC	AATGCTGTAT	1560
TGCCTCCTAA	GTGCAAGGGC	CATCACTGAA	CAGGAGTGCA	ATGCTGTGAA	ACAGAAACCA	1620
CACACCTTAC	AAGCAAGCAC	ACTGATTGAT	ACTGTGTTAG	CAAAAGGAAA	CACTGCAGCA	1680
ACCTCATTCA	GAAACTCCCT	TCGGGAAATT	GACCCTGCGT	TATACAGAGA	TATATTTGTG	1740
CAACAGGACA	TTAGGAGTCT	TCCCACAGAT	GACATTGCAG	CTCTACCAAT	GGAAGAACAG	1800
TTGCGGCCCC	TCCCGGAGGA	CAGAATGTGT	AAAGTGTGTA	TGGACCGAGA	GGTATCCATC	1860
GTGTTTCAATC	CCTGTGGCCA	TCTGGTCGTG	TGCAAAGACT	GCGCTCCCTC	TCTGAGGAAG	1920
TGTCCCATCT	GTAGAGGGAC	CATCAAGGGC	ACAGTGCGCA	CATTTCTCTC	CTGAACAAGA	1980
CTAATGGTCC	ATGGCTGCAA	CTTCAGCCAG	GAGGAAGTTC	ACTGTCACTC	CCAGTTCCAT	2040
TCGGAACCTG	AGGCCAGCCT	GGATAGCACG	AGACACCGCC	AAACACACAA	ATATAAACAT	2100
GAAAACTTT	TGTCTGAAGT	CAAGAATGAA	TGAATTACTT	ATATAATAAT	TTTAATTGGT	2160
TTCTTAAAA	GTGCTATTTG	TTCCCAACTC	AGAAAATTGT	TTTCTGTAAA	CATATTTACA	2220
TACTACCTGC	ATCTAAAGTA	TTCATATATT	CATATATTCA	GATGTCATGA	GAGAGGGTTT	2280
TGTTCTTGTT	CCTGAAAAGC	TGGTTTATCA	TCTGATCAGC	ATATACTGCG	CAACGGGCAG	2340

- 97 -

GGCTAGAATC CATGAACCAA GCTGCAAAGA TCTCACGCTA AATAAGGCGG AAAGATTTGG 2400
 AGAAACGAAA GGAAATTCTT TCCTGTCCAA TGTATACTCT TCAGACTAAT 2460
 TATCAAGCCT 2474

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 602 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met	Asn	Met	Val	Gln	Asp	Ser	Ala	Phe	Leu	Ala	Lys	Leu	Met	Lys	Ser	1	5	10	15
Ala	Asp	Thr	Phe	Glu	Leu	Lys	Tyr	Asp	Phe	Ser	Cys	Glu	Leu	Tyr	Arg	20	25	30	
Leu	Ser	Thr	Tyr	Ser	Ala	Phe	Pro	Arg	Gly	Val	Pro	Val	Ser	Glu	Arg	35	40	45	
Ser	Leu	Ala	Arg	Ala	Gly	Phe	Tyr	Tyr	Thr	Gly	Ala	Asn	Asp	Lys	Val	50	55	60	
Lys	Cys	Phe	Cys	Cys	Gly	Leu	Met	Leu	Asp	Asn	Trp	Lys	Gln	Gly	Asp	65	70	75	80
Ser	Pro	Met	Glu	Lys	His	Arg	Lys	Leu	Tyr	Pro	Ser	Cys	Asn	Phe	Val	85	90	95	
Gln	Thr	Leu	Asn	Pro	Ala	Asn	Ser	Leu	Glu	Ala	Ser	Pro	Arg	Pro	Ser	100	105	110	
Leu	Pro	Ser	Thr	Ala	Met	Ser	Thr	Met	Pro	Leu	Ser	Phe	Ala	Ser	Ser	115	120	125	
Glu	Asn	Thr	Gly	Tyr	Phe	Ser	Gly	Ser	Tyr	Ser	Ser	Phe	Pro	Ser	Asp	130	135	140	
Pro	Val	Asn	Phe	Arg	Ala	Asn	Gln	Asp	Cys	Pro	Ala	Leu	Ser	Thr	Ser	145	150	155	160
Pro	Tyr	His	Phe	Ala	Met	Asn	Thr	Glu	Lys	Ala	Arg	Leu	Leu	Thr	Tyr	165	170	175	
Glu	Thr	Trp	Pro	Leu	Ser	Phe	Leu	Ser	Pro	Ala	Lys	Leu	Ala	Lys	Ala	180	185	190	
Gly	Phe	Tyr	Tyr	Ile	Gly	Pro	Gly	Asp	Arg	Val	Ala	Cys	Phe	Ala	Cys	195	200	205	
Asp	Gly	Lys	Leu	Ser	Asn	Trp	Glu	Arg	Lys	Asp	Asp	Ala	Met	Ser	Glu	210	215	220	

- 98 -

His Gln Arg His Phe Pro Ser Cys Pro Phe Leu Lys Asp Leu Gly Gln
 225 230 235 240
 Ser Ala Ser Arg Tyr Thr Val Ser Asn Leu Ser Met Gln Thr His Ala
 245 250 255
 Ala Arg Ile Arg Thr Phe Ser Asn Trp Pro Ser Ser Ala Leu Val His
 260 265 270
 Ser Gln Glu Leu Ala Ser Ala Gly Phe Tyr Tyr Thr Gly His Ser Asp
 275 280 285
 Asp Val Lys Cys Leu Cys Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser
 290 295 300
 Gly Asp Asp Pro Trp Val Glu His Ala Lys Trp Phe Pro Arg Cys Glu
 305 310 315 320
 Tyr Leu Leu Arg Ile Lys Gly Gln Glu Phe Val Ser Gln Val Gln Ala
 325 330 335
 Gly Tyr Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Ser Pro
 340 345 350
 Glu Asp Glu Asn Ala Asp Ala Ala Ile Val His Phe Gly Pro Gly Glu
 355 360 365
 Ser Ser Glu Asp Val Val Met Met Ser Thr Pro Val Val Lys Ala Ala
 370 375 380
 Leu Glu Met Gly Phe Ser Arg Ser Leu Val Arg Gln Thr Val Gln Trp
 385 390 395 400
 Gln Ile Leu Ala Thr Gly Glu Asn Tyr Arg Thr Val Ser Asp Leu Val
 405 410 415
 Ile Gly Leu Leu Asp Ala Glu Asp Glu Met Arg Glu Glu Gln Met Glu
 420 425 430
 Gln Ala Ala Glu Glu Glu Glu Ser Asp Asp Leu Ala Leu Ile Arg Lys
 435 440 445
 Asn Lys Met Val Leu Phe Gln His Leu Thr Cys Val Thr Pro Met Leu
 450 455 460
 Tyr Cys Leu Leu Ser Ala Arg Ala Ile Thr Glu Gln Glu Cys Asn Ala
 465 470 475 480
 Val Lys Gln Lys Pro His Thr Leu Gln Ala Ser Thr Leu Ile Asp Thr
 485 490 495
 Val Leu Ala Lys Gly Asn Thr Ala Ala Thr Ser Phe Arg Asn Ser Leu
 500 505 510
 Arg Glu Ile Asp Pro Ala Leu Tyr Arg Asp Ile Phe Val Gln Gln Asp
 515 520 525
 Ile Arg Ser Leu Pro Thr Asp Asp Ile Ala Ala Leu Pro Met Glu Glu
 530 535 540
 Gln Leu Arg Pro Leu Pro Glu Asp Arg Met Cys Lys Val Cys Met Asp
 545 550 555 560

- 99 -

Arg Glu Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val Val Cys
565 570 575

Lys Asp Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg Gly Thr
580 585 590

Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
595 600

(2). INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2416 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CTGTGGTGGG	GATCTATTGT	CCAAGTGGTG	AGAACTTCA	TCTGGAAGTT	TAAGCGGTCA	60
GAAATACTAT	TACTACTCAT	GGACAAAACT	GTCTCCCAGA	GACTCGCCCA	AGGTACCTTA	120
CACCCAAAAA	CTTAAACGTA	TAATGGAGAA	GAGCACAATC	TTGTCAAATT	GGACAAAGGA	180
GAGCGAAGAA	AAAATGAAGT	TTGACTTTTC	GTGTGAACTC	TACCGAATGT	CTACATATTC	240
AGCTTTTCCC	AGGGGAGTTC	CTGTCTCAGA	GAGGAGTCTG	GCTCGTGCTG	GCTTTTATTA	300
TACAGGTGTG	AATGACAAAG	TCAAGTGCTT	CTGCTGTGGC	CTGATGTTGG	ATAACTGGAA	360
ACAAGGGGAC	AGTCCTGTTG	AAAAGCACAG	ACAGTTCTAT	CCCAGCTGCA	GCTTTGTACA	420
GACTCTGCTT	TCAGCCAGTC	TGCAGTCTCC	ATCTAAGAAT	ATGTCTCCTG	TGAAAAGTAG	480
ATTTGCACAT	TCGTACCTC	TGGAACGAGG	TGGCATTAC	TCCAACCTGT	GCTCTAGCCC	540
TCTTAATTCT	AGAGCAGTGG	AAGACTTCTC	ATCAAGGATG	GATCCCTGCA	GCTATGCCAT	600
GAGTACAGAA	GAGGCCAGAT	TTCTTACTTA	CAGTATGTGG	CCTTTAAGTT	TTCTGTCAAC	660
AGCAGAGCTG	GCCAGAGCTG	GCTTCTATTA	CATAGGGCCT	GGAGACAGGG	TGGCCTGTTT	720
TGCCTGTGGT	GGGAACTGA	GCAACTGGGA	ACCAAAGGAT	TATGCTATGT	CAGAGCACCG	780
CAGACATTTT	CCCCACTGTC	CATTTCTGGA	AAATACTTCA	GAAACACAGA	GGTTTAGTAT	840
ATCAAATCTA	AGTATGCAGA	CACACTCTGC	TCGATTGAGG	ACATTTCTGT	ACTGGCCACC	900
TAGTGTTTCT	GTTTCTGCTG	AGCAGCTTGC	AAGTGCTGGA	TTCTATTACG	TGGATCGCAA	960
TGATGATGTC	AAGTGCTTTT	GTTGTGATGG	TGGCTTGAGA	TGTTGGGAAC	CTGGAGATGA	1020
CCCCTGGATA	GAACACGCCA	AATGGTTTCC	AAGGTGTGAG	TTCTTGATAC	GGATGAAGGG	1080
TCAGGAGTTT	GTTGATGAGA	TTCAAGCTAG	ATATCCTCAT	CTTCTTGAGC	AGCTGTTGTC	1140
CACTTCAGAC	ACCCAGGAG	AAGAAAATGC	TGACCCTACA	GAGACAGTGG	TGCATTTTGG	1200

- 100 -

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CCCTGGAGAA AGTTCGAAAG ATGTCGTCAT GATGAGCAGC CCTGTGGTTA AAGCAGCCTT 1260
GGAAATGGGC TTCAGTAGGA GCCTGGTGAG ACAGACGGTT CAGCGGCAGA TCCTGGCCAC 1320
TGGTGAGAAC TACAGGACCG TCAATGATAT TGTCTCAGTA CTTTGAATG CTGAAGATGA 1380
GAGAAGAGAA GAGGAGAAGG AAAGACAGAC TGAAGAGATG GCATCAGGTG ACTTATCACT 1440
GATTCGGAAG AATAGAATGG CCCTCTTTCA ACAGTTGACA CATGTCCTTC CTATCCTGGA 1500
TAATCTTCTT GAGGCCAGTG TAATTACAAA ACAGGAACAT GATATTATTA GACAGAAAAC 1560
ACAGATACCC TTACAAGCAA GAGAGCTTAT TGACACCGTT TTAGTCAAGG GAAATGCTGC 1620
AGCCAACATC TTCAAAAACCT CTCTGAAGGG AATTGACTCC ACGTTATATG AAAACTTATT 1680
TGTGGAAGAA AATATGAAGT ATATTCCAAC AGAAGACGTT TCAGGCTTGT CATTGGAAGA 1740
GCAGTTGCGG AGATTACAAG AAGAACGAAC TTGCAAAGTG TGTATGGACA GAGAGGTTTC 1800
TATTGTGTTT ATTCCGTGTG GTCATCTAGT AGTCTGCCAG GAATGTGCCC CTTCTCTAAG 1860
GAAGTGCCCC ATCTGCAGGG GGACAATCAA GGGGACTGTG CGCACATTTT TCTCATGAGT 1920
GAAGAATGGT CTGAAAGTAT TGTGGACAT CAGAAGCTGT CAGAACAAAG AATGAACTAC 1980
TGATTTTCAGC TCTTCAGCAG GACATTCTAC TCTCTTTCAA GATTAGTAAT CTTGCTTTAT 2040
GAAGGGTAGC ATTGTATATT TAAGCTTAGT CTGTTGCAAG GGAAGGTCTA TGCTGTTGAG 2100
CTACAGGACT GTGTCTGTTT CAGAGCAGGA GTTGGGATGC TTGCTGTATG TCCTTCAGGA 2160
CTTCTTGGA TTTGGGAATT TGGGGAAAGC TTTGGAATCC AGTGATGTGG AGCTCAGAAA 2220
TCCTGGAACC AGTGAAGCTG GTACTCAGTA GATAGGGTAC CCTGTACTTC TTGGTGCTTT 2280
TCCAGTCTGG GAAATAAGGA GGAATCTGCT GCTGGTAAAA ATTTGCTGGA TGTGAGAAAT 2340
AGATGAAAGT GTTTCGGGTG GGGGCGTGCA TCAGTGTAGT GTGTGCAGGG ATGTATGCAG 2400
GCCAAACACT GTGTAG 2416

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(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 591 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

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Met Glu Lys Ser Thr Ile Leu Ser Asn Trp Thr Lys Glu Ser Glu Glu
1           5           10           15
Lys Met Lys Phe Asp Phe Ser Cys Glu Leu Tyr Arg Met Ser Thr Tyr
20           25           30

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- 101 -

Ser Ala Phe Pro Arg Gly Val Pro Val Ser Glu Arg Ser Leu Ala Arg
 35 40 45
 Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys Val Lys Cys Phe Cys
 50 55 60
 Cys Gly Leu Met Leu Asp Asn Trp Lys Gln Gly Asp Ser Pro Val Glu
 65 70 75 80
 Lys His Arg Gln Phe Tyr Pro Ser Cys Ser Phe Val Gln Thr Leu Leu
 85 90 95
 Ser Ala Ser Leu Gln Ser Pro Ser Lys Asn Met Ser Pro Val Lys Ser
 100 105 110
 Arg Phe Ala His Ser Ser Pro Leu Glu Arg Gly Gly Ile His Ser Asn
 115 120 125
 Leu Cys Ser Ser Pro Leu Asn Ser Arg Ala Val Glu Asp Phe Ser Ser
 130 135 140
 Arg Met Asp Pro Cys Ser Tyr Ala Met Ser Thr Glu Glu Ala Arg Phe
 145 150 155 160
 Leu Thr Tyr Ser Met Trp Pro Leu Ser Phe Leu Ser Pro Ala Glu Leu
 165 170 175
 Ala Arg Ala Gly Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys
 180 185 190
 Phe Ala Cys Gly Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Tyr Ala
 195 200 205
 Met Ser Glu His Arg Arg His Phe Pro His Cys Pro Phe Leu Glu Asn
 210 215 220
 Thr Ser Glu Thr Gln Arg Phe Ser Ile Ser Asn Leu Ser Met Gln Thr
 225 230 235 240
 His Ser Ala Arg Leu Arg Thr Phe Leu Tyr Trp Pro Pro Ser Val Pro
 245 250 255
 Val Gln Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Asp Arg
 260 265 270
 Asn Asp Asp Val Lys Cys Leu Cys Cys Asp Gly Gly Leu Arg Cys Trp
 275 280 285
 Glu Pro Gly Asp Asp Pro Trp Ile Glu His Ala Lys Trp Phe Pro Arg
 290 295 300
 Cys Glu Phe Leu Ile Arg Met Lys Gly Gln Glu Phe Val Asp Glu Ile
 305 310 315 320
 Gln Ala Arg Tyr Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp
 325 330 335
 Thr Pro Gly Glu Glu Asn Ala Asp Pro Thr Glu Thr Val Val His Phe
 340 345 350
 Gly Pro Gly Glu Ser Ser Lys Asp Val Val Met Met Ser Thr Pro Val
 355 360 365

- 102 -

Val Lys Ala Ala Leu Glu Met Gly Phe Ser Arg Ser Leu Val Arg Gln
 370 375 380
 Thr Val Gln Arg Gln Ile Leu Ala Thr Gly Glu Asn Tyr Arg Thr Val
 385 390 395 400
 Asn Asp Ile Val Ser Val Leu Leu Asn Ala Glu Asp Glu Arg Arg Glu
 405 410 415
 Glu Glu Lys Glu Arg Gln Thr Glu Glu Met Ala Ser Gly Asp Leu Ser
 420 425 430
 Leu Ile Arg Lys Asn Arg Met Ala Leu Phe Gln Gln Leu Thr His Val
 435 440 445
 Leu Pro Ile Leu Asp Asn Leu Leu Glu Ala Ser Val Ile Thr Lys Gln
 450 455 460
 Glu His Asp Ile Ile Arg Gln Lys Thr Gln Ile Pro Leu Gln Ala Arg
 465 470 475 480
 Glu Leu Ile Asp Thr Val Leu Val Lys Gly Asn Ala Ala Ala Asn Ile
 485 490 495
 Phe Lys Asn Ser Leu Lys Gly Ile Asp Ser Thr Leu Tyr Glu Asn Leu
 500 505 510
 Phe Val Glu Lys Asn Met Lys Tyr Ile Pro Thr Glu Asp Val Ser Gly
 515 520 525
 Leu Ser Leu Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Arg Thr Cys
 530 535 540
 Lys Val Cys Met Asp Arg Glu Val Ser Ile Val Phe Ile Pro Cys Gly
 545 550 555 560
 His Leu Val Val Cys Gln Glu Cys Ala Pro Ser Leu Arg Lys Cys Pro
 565 570 575
 Ile Cys Arg Gly Thr Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
 580 585 590

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
 1 5 10

(2) INFORMATION FOR SEQ ID NO:44:

- 103 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

AGTGCGGGTT TTTATTATGT G 21

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AGATGACCAC AAGGAATAAA CACTA 25

- 104 -

What is claimed is:

1. A substantially pure nucleic acid encoding an IAP polypeptide.

2. The nucleic acid of claim 1, wherein said
5 polypeptide comprises a ring zinc finger domain and at least one BIR domain.

3. The nucleic acid of claim 2, wherein said polypeptide has at least two BIR domains.

4. The nucleic acid of claim 3, wherein said
10 polypeptide has at least three BIR domains.

5. The nucleic acid of claim 1, wherein said polypeptide comprises at least one BIR domain but lacks a ring zinc finger domain.

6. The nucleic acid of claim 5, wherein said
15 polypeptide has at least two BIR domains.

7. The nucleic acid of claim 6, wherein said polypeptide has at least three BIR domains.

8. The nucleic acid of claim 1, wherein said polypeptide comprises a ring zinc finger domain but lacks
20 a BIR domain.

9. The nucleic acid of claim 1, wherein said nucleic acid is mammalian.

10. The nucleic acid of claim 9, wherein said mammal is a human.

- 105 -

11. The nucleic acid of claim 9, wherein said DNA contains the m-xiap gene, the m-hiap-1 gene, or the m-hiap-2 gene.

12. The nucleic acid of claim 10, wherein said
5 DNA contains the xiap gene, the hiap-1 gene, or the hiap-2 gene.

13. The nucleic acid of claim 1, wherein said nucleic acid is genomic DNA or cDNA.

14. A substantially pure DNA having the
10 sequence of Fig. 1, or degenerate variants thereof, and encoding the amino acid sequence of Fig. 1, the sequence of Fig. 2, or degenerate variants thereof, and encoding the amino acid sequence of Fig. 2, the sequence of Fig. 3, or degenerate variants thereof, and encoding the amino
15 acid sequence of Fig. 3, or the sequence of Fig. 4, or degenerate variants thereof, and encoding the amino acid sequence of Fig. 4.

15. Substantially pure DNA having about 50% or greater nucleotide sequence identity to the DNA sequence
20 of Fig. 1, Fig. 2, Fig. 3, or Fig. 4.

16. A purified DNA sequence substantially identical to the DNA sequence shown in Fig. 1, Fig. 2, Fig. 3, or Fig. 4.

17. The DNA of claim 1, wherein said DNA is
25 operably linked to regulatory sequences for expression of said polypeptide and wherein said regulatory sequences comprise a promoter.

- 106 -

18. The DNA of claim 17, wherein said promoter is a constitutive promoter, is inducible by one or more external agents, or is cell-type specific.

19. A vector comprising the DNA of claim 1,
5 said vector being capable of directing expression of the peptide encoded by said DNA in a vector-containing cell.

20. A cell that contains the DNA of claim 1.

21. The cell of claim 20, said cell being
10 present in a patient having a disease that is caused by excessive or insufficient cell death.

22. The cell of claim 20, said cell being
selected from the group consisting of a fibroblast, a neuron, a glial cell, an insect cell, an embryonic stem cell, and a lymphocyte.

15 23. A transgenic cell that contains the DNA of claim 1, wherein said DNA is expressed in said transgenic cell.

24. A transgenic animal generated from the cell
20 of claim 20, wherein said DNA is expressed in said transgenic animal.

25. A substantially pure mammalian IAP polypeptide, or fragment thereof.

26. The polypeptide of claim 25, said
25 polypeptide being encoded by the nucleic acid of claim 5, claim 6, claim 7, or claim 8.

- 107 -

27. The polypeptide of claim 25, said polypeptide comprising an amino acid sequence substantially identical to an amino acid sequence shown in Fig. 1, Fig. 2, Fig. 3, or Fig. 4.

5 28. The polypeptide of claim 25, wherein said polypeptide is a mammalian polypeptide.

29. The polypeptide of claim 25, wherein said polypeptide is a human polypeptide.

10 30. The polypeptide of claim 28, wherein said polypeptide is M-XIAP, M-HIAP-1, or M-HIAP-2.

31. The polypeptide of claim 29, wherein said polypeptide is XIAP, HIAP-1, or HIAP-2.

15 32. A therapeutic composition comprising as an active ingredient an IAP polypeptide according to claim 25, said active ingredient being formulated in a physiologically acceptable carrier.

33. The composition of claim 32, said active ingredient being an IAP polypeptide encoded by the nucleic acid of claim 5, claim 6, claim 7, or claim 8.

20 34. A method of inhibiting apoptosis in a cell, said method comprising administering to said cell an apoptosis inhibiting amount of IAP polypeptide.

35. The method of claim 34, wherein said cell is in a mammal.

25 36. The method of claim 35, wherein said mammal is a human.

- 108 -

37. The method of claim 35, wherein said human has been diagnosed as being HIV-positive, or as having AIDS, a neurodegenerative disease, a myelodysplastic syndrome, or an ischemic injury.

5 38. The method of claim 37, wherein said ischemic injury is caused by a myocardial infarction, a stroke, a reperfusion injury, or a toxin-induced liver disease.

10 39. A method of inhibiting apoptosis in a mammal, said method comprising providing a transgene encoding an IAP polypeptide or fragment thereof to a cell of said mammal, said transgene being positioned for expression in said cell.

15 40. The method of claim 39 wherein said transgene encodes M-XIAP, M-HIAP-1, or M-HIAP-2.

41. The method of claim 39, wherein said mammal is a human.

42. The method of claim 41, wherein said polypeptide is XIAP, HIAP-1, or HIAP-2.

20 43. The method of claim 39, wherein said mammal is HIV-positive or has AIDS.

44. The method of claim 43, wherein said cell is a T cell.

25 45. The method of claim 44, wherein said T cell is a CD4⁺ T cell.

- 109 -

46. The method of claim 39, wherein said mammal has a neurodegenerative disease.

47. The method of claim 39, wherein said mammal has an ischemic injury.

5 48. The method of claim 47, wherein said ischemic injury is caused by a myocardial infarction, a stroke, a reperfusion injury, or a toxin-induced liver disease.

10 49. A method of detecting an IAP gene in an animal cell, said method comprising contacting the DNA of claim 2, or a portion thereof that is greater than about 18 nucleotides in length, with a preparation of genomic DNA from said animal cell, said method providing detection of DNA sequences having about 50% or greater
15 nucleotide sequence identity with the sequence of Fig. 1, Fig. 2, Fig. 3, or Fig. 4.

50. A method of obtaining an IAP polypeptide, said method comprising:

20 (a) providing a cell with DNA encoding an IAP polypeptide, said DNA being positioned for expression in said cell;

(b) culturing said cell under conditions for expressing said DNA; and

25 (c) isolating said IAP polypeptide.

51. The method of claim 50, wherein said DNA further comprises a promotor inducible by one or more external agents.

- 110 -

52. The method of claim 45 wherein said IAP polypeptide is XIAP, HIAP-1, HIAP-2, M-XIAP, M-HIAP-1, or M-HIAP-2.

53. A method of isolating an IAP gene or
5 portion thereof having sequence identity to xiap, m-xiap, hiap-1, m-hiap-1, hiap-2, or m-hiap-2, said method comprising amplifying by PCR said IAP gene or portion thereof using oligonucleotide primers wherein said primers

10 (a) are each greater than 13 nucleotides in length;

(b) each have regions of complementarily to opposite DNA strands in a region of the nucleotide sequence of either Fig. 1, Fig. 2, Fig. 3, or Fig. 4; and

15 (c) optionally contain sequences capable of producing restriction enzyme cut sites in the amplified product; and isolating said IAP gene or portion thereof.

54. A substantially pure polypeptide comprising a ring zinc finger domain having the sequence: Glu-Xaa1-
20 Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa2-Xaa1-Xaa1-Xaa1-Cys-Lys-Xaa3-Cys-Met-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa3-Xaa1-Phe-Xaa1-Pro-Cys-Gly- His-Xaa1-Xaa1-Xaa1-Cys-Xaa1-Xaa1-Cys-Ala-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Cys-Pro-Xaa1-Cys, wherein Xaa1 is any amino acid, Xaa2 is Glu or Asp and Xaa3 is Val or
25 Ile.

55. The polypeptide of claim 54, further comprising at least one BIR domain having a copy of the sequence: Xaa1-Xaa1-Xaa1-Arg-Leu-Xaa1-Thr-Phe-Xaa1-Xaa1-Trp- Pro-Xaa2-Xaa1-Xaa1-Xaa2-Xaa2-Xaa1-Xaa1-Xaa1-Xaa1-
30 Leu-Ala- Xaa1-Ala-Gly-Phe-Tyr-Tyr-Xaa1-Gly-Xaa1-Xaa1-Asp-Xaa1-Val-Xaa1-Cys-Phe-Xaa1-Cys-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Trp-Xaa1 Xaa1-Xaa1-Asp-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-His-

- 111 -

Xaa1-Xaa1-Xaa1-Xaa1-Pro-Xaa1-Cys-Xaa1-Phe-Val, wherein Xaa1 may be any amino acid and Xaa2 may be any amino acid or may be absent.

56. The polypeptide of claim 55, said
5 polypeptide comprising at least two of said BIR domains.

57. The polypeptide of claim 56, said polypeptide comprising at least three of said BIR domains.

58. A recombinant IAP gene encoding the
10 polypeptide of claim 54.

59. A method of isolating an IAP gene or fragment thereof from a cell, said method comprising:
(a) providing a sample of cellular DNA;
(b) providing a pair of oligonucleotides having
15 sequence homology to a conserved region of an IAP gene;
(c) combining said pair of oligonucleotides with said cellular DNA sample under conditions suitable for polymerase chain reaction-mediated DNA amplification; and
(d) isolating said amplified IAP gene or
20 fragment thereof.

60. The method of claim 59, wherein said amplification is carried out using a reverse-transcription polymerase chain reaction.

61. The method of claim 60, wherein said
25 reverse-transcription polymerase chain reaction is RACE.

62. A method of identifying an IAP gene in a mammalian cell, said method comprising:

- 112 -

(a) providing a preparation of mammalian cellular DNA;

(b) providing a detectably-labelled DNA sequence having homology to a conserved region of an IAP gene;

5 (c) contacting said preparation of cellular DNA with said detectably-labelled DNA sequence under hybridization conditions that provide detection of genes having 50% or greater nucleotide sequence identity; and

10 (d) identifying an IAP gene by its association with said detectable label.

63. The method of claim 62, wherein said DNA sequence is produced according to the method of claim 53.

64. A method of isolating an IAP gene from a recombinant DNA library, said method comprising:

15 (a) providing a recombinant DNA library;

(b) contacting said recombinant DNA library with a detectably-labelled gene fragment produced according to the method of claim 49 under hybridization conditions that provide for detection of genes having 50% or greater
20 nucleotide sequence identity; and

(c) isolating a member of an IAP gene by its association with said detectable label.

65. A method of isolating an IAP gene from a recombinant DNA library, said method comprising:

25 (a) providing a recombinant DNA library;

(b) contacting said recombinant DNA library with a detectably-labelled oligonucleotide of any of claim 49 under hybridization conditions that provide for detection of genes having 50% or greater nucleotide sequence
30 identity; and

(c) isolating an IAP gene by its association with said detectable label.

- 113 -

66. A recombinant mammalian polypeptide capable of inhibiting apoptosis wherein said polypeptide comprises a ring zinc finger sequence: Glu-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa2-Xaa1-Xaa1-Xaa1-Cys-Lys-Xaa3-Cys-Met-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa3-Xaa1-Phe-Xaa1-Pro-Cys-Gly-His-Xaa1-Xaa1- Xaa1-Cys-Xaa1-Xaa1-Cys-Ala-Xaa1-Xaa1-Xaa1-Xaa1-Cys-Pro Xaa1-Cys, wherein Xaa1 and amino acid, Xaa2 is Glu or Asp and Xaa3 is Val or Ile;

and at least one BIR domain having the sequence
10 Xaa1-Xaa1-Xaa1-Arg-Leu-Xaa1-Thr-Phe-Xaa1-Xaa1-Trp-Pro-Xaa2- Xaa1-Xaa1-Xaa2-Xaa2-Xaa1-Xaa1-Xaa1-Xaa1-Leu-Ala-Xaa1-Ala-Gly-Phe-Tyr-Tyr-Xaa1-Gly-Xaa1-Xaa1-Asp-Xaa1-Val-Xaa1-Cys-Phe-Xaa1-Cys-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Trp-Xaa1-Xaa1- Xaa1-Asp-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-His-Xaa1-
15 Xaa1-Xaa1-Xaa1- Pro-Xaa1-Cys-Xaa1-Phe-Val, wherein Xaa1 may be any amino acid and Xaa2 is any amino acid or is absent.

67. An IAP gene isolated according to a method comprising:

- 20 (a) providing a sample of cellular DNA;
(b) providing a pair of oligonucleotides having sequence homology to a conserved region of an IAP disease-resistance gene;
(c) combining said pair of oligonucleotides with
25 said cellular DNA sample under conditions suitable for polymerase chain reaction-mediated DNA amplification; and
(d) isolating said amplified IAP gene or fragment thereof.

68. An IAP gene isolated according to the method comprising:

- 30 (a) providing a preparation of cellular DNA;
(b) providing a detectably-labelled DNA sequence having homology to a conserved region of an IAP gene;

- 114 -

(c) contacting said preparation of cellular DNA with said detectably-labelled DNA sequence under hybridization conditions providing detection of genes having 50% or greater nucleotide sequence identity; and

5 (d) identifying an IAP gene by its association with said detectable label.

69. A method of identifying an IAP gene, said method comprising:

- 10 (a) providing a mammalian cell sample;
(b) introducing by transformation into said cell sample a candidate IAP gene;
(c) expressing said candidate IAP gene within said cell sample; and
(d) determining whether said sample exhibits an
15 altered level of apoptosis whereby an alteration in the level of apoptosis identifies an IAP gene.

70. The method of claim 69, wherein said cell sample is selected from the group consisting of a lymphocyte, a fibroblast, an insect cell, a glial cell,
20 an embryonic stem cell, and a neuron.

71. The method of claim 69, wherein said candidate IAP gene is obtained from a cDNA expression library.

25 72. An IAP gene isolated according to the method comprising:

- (a) providing a cell sample;
(b) introducing by transformation into said cell sample a candidate IAP gene;
(c) expressing said candidate IAP gene within
30 said cell sample; and

- 115 -

(d) determining whether said cell sample exhibits a decreased apoptosis response, whereby a decreased level of apoptosis identifies an IAP gene.

73. A purified antibody that binds specifically
5 to an IAP family polypeptide.

74. A method of identifying a compound that modulates apoptosis, said method comprising:

(a) providing a cell expressing an IAP polypeptide; and

10 (b) contracting said cell with a candidate compound and monitoring the expression of an IAP gene, an alteration in the level of expression of said gene indicating the presence of a compound which modulates apoptosis.

15 75. The method of claim 74, wherein said IAP gene is xiap, hiap-1, hiap-2, m-xiap, m-hiap-1, or m-hiap-2.

76. The method of claim 74, wherein said cell
20 is a lymphocyte, said IAP is selected from the group consisting of hiap-1 and hiap-2, and said modulating is an increase in hiap-1 or hiap-2 expression.

77. A method of diagnosing a mammal for the presence of an apoptosis disease or an increased likelihood of developing a disease involving apoptosis in
25 a mammal, said method comprising isolating a sample of nucleic acid from said mammal and determining whether said nucleic acid comprises an IAP mutation, said mutation being an indication that said mammal has an apoptosis disease or an increased likelihood of
30 developing a disease involving apoptosis.

- 116 -

78. A method of diagnosing a mammal for the presence of an apoptosis disease or an increased likelihood of developing an apoptosis disease, said method comprising measuring IAP gene expression in a
5 sample from said mammal, an alteration in said expression relative to a sample from an unaffected mammal being an indication that said mammal has an apoptosis disease or increased likelihood of developing an apoptosis disease.

79. The method of claim 77 or 78, wherein said
10 IAP gene is xiap, hiap-1, hiap-2, m-xiap, m-hiap-1, or m-hiap-2.

80. The method of claim 77 or 78, wherein said gene expression is measured by assaying the amount of IAP polypeptide in said sample.

15 81. The method of claim 80, wherein said IAP polypeptide is measured by immunological methods or by assaying the amount of IAP RNA in said sample.

82. A kit for diagnosing a mammal for the presence of an apoptosis disease of an increased
20 likelihood of developing an apoptosis disease, said kit comprising a substantially pure antibody that specifically binds an IAP polypeptide.

83. The kit of claim 82, further comprising a means for detecting said binding of said antibody to said
25 IAP polypeptide.

84. The method of claim 34, said method comprising administering to said cell an apoptosis inhibiting amount of the polypeptide of claim 8.

- 117 -

85. A method of inducing apoptosis in a cell, said method comprising administering to said cell a negative regulator of the IAP-dependent anti-apoptotic pathway.

5 86. The method of claim 85, wherein said negative regulator is an IAP polypeptide comprising a ring zinc finger, but lacking at least one BIR domain.

 87. The method of claim 85, wherein said cell is transfected with a gene encoding the IAP polypeptide
10 of claim 8.

 88. The method of claim 85, wherein said negative regulator is a purified antibody or a fragment thereof that binds specifically to an IAP polypeptide.

 89. The method of claim 88, wherein said
15 antibody specifically binds an approximately 26 kDa cleavage product of an IAP polypeptide, said cleavage product comprising at least one BIR domain but lacking a ring zinc finger domain

 90. The method of claim 85, wherein said
20 negative regulator is an IAP antisense mRNA molecule.

 91. An IAP nucleic acid for use in modulating apoptosis.

 92. An IAP polypeptide for use in modulating apoptosis.

25 93. The use of an IAP polypeptide for the manufacture of a medicament for the modulation of apoptosis.

- 118 -

94. The use of an IAP nucleic acid for the manufacture of a medicament for the modulation of apoptosis.

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FIG. 2 (pg. 1 of 7)

mouse xiap

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FIG. 4 (pp. 1 of 7)

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SEQ ID NO:39      GAATTCGGGAGACCTACACCCCCGGAGATCAGAGGTCATTGCTGGCGTTTCAGAGCCCTAG
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      GAAGTGGGGTGCGGTATCAGCCTAGCAGTAAAACCGSACCAGAAGCCATGCACAAAACCTAC
61 ..... 120
      ATCCCCAGAGAAAGACTTGTCCTTTCCGCTGCTCTCTATCTCAGCATGAACATGGTTTCAA
121 ..... 180
SEQ ID NO:40      M N M V Q
      SACAGCCGCTTTCTAGCCCAAGCTTATGAAGAGTGCCTGACACGCTTTGAGTTGAAGTATGAC
181 ..... 240
      D S A F L A K L M K S A D T F E L K Y D
      TTTTCCTGTGAGCTGTACGGATTGTCCAGCTATTGAGCTTTTCCAGGGGAGTTCCTGTG
241 ..... 300
      F S C E L Y R L S T Y S A F P R G V P V
      TCAGAAAGGAGTCTGGCTGCTGCTGCTGCTTTTACTACACTGCTGCCATGACAAAGTCAAG
301 ..... 360
      S E R S L A R A G F Y Y T G A N D K V K
      TGCTTCTGCTGTGGCCTGATGCTAGACAACCTGGAAGCAAGGGGACAGTCCCATGGAGAAG
361 ..... 420
      C F C C G L M L D N W K Q G D S P M E K
      CACAGAAAGTTGTACCCGAGCTGCACTTTTGTACAGACTTTTGAATTCAGCCAACAGTCTG
421 ..... 480
      H R K L Y P S C N F V Q T L N P A N S L
      GAAGCTAGTCTGCTGGCGCTTCTGCTGCTTCCAGCCGATGAGCACCATGCGCTTTGAGCTTT
481 ..... 540
      E A S P R P S L P S T A M S T M P L S F
      GCAAGTTTGTGAGAACTAGTGGCTATTTGAGTGGCTTTTACTGAGGTTTCCCTGAGACTCT
541 ..... 600
      A S S E N T G Y F S G S Y S F P S D P
      GTGAAGTCCCGAGCAAAATCAAGATTGCTGCTTTGAGCAGAAAGTCCCTACCACTTTTCA
601 ..... 660
      Y N P R A M Q D I F A L E T S P Y H P A
      ATGAACACAGAGAAAGCCGAGATTACTCACTATGAACATGGCCATTGTCTTTTCTGTCA
661 ..... 720
      M N T E R A R L L T Y E T W P L S F L S
      CCAGCAAAAGCTGGCCAAAGCAGGCTTTTACTACATAGGACCTGGAGATAGAGTGGCCTGC
721 ..... 780
      P A K L A K A G F Y Y I G F D D R V A C

```

Fig. 5 (page 1 of 3)

10/35

781	TTTTCCTCCGATGGGAAACTGAGCAACTGGGAACTAAGGATGATGCATCTTCAGAGGAC	840
	F A C D G K L S N W E R K D D A M S E H	
841	CAGAGGCGATTTCCCCAGCTGTCCGTTCTTAAAAGACTTGGGTCAGTCTGCTTCGAGATAC	900
	C R H F P S C P F L K D L D I S A S R Y	
901	ACTGTCTCTAACCTGAGCATTCGAGACACACCCAGCCCTTATTAGAAGATTCTCTAACTGG	960
	T V S N L S M C T H A A R I R T F S N W	
961	CTTCTTACTGGACTAGTTCATTTCGAGAACTTSCAAAGTCCGGGCTTTTATTATACAGGA	1020
	P S S A L V H S Q E L A S A G F Y Y T G	
1021	CACAGTGATGATGTCAAGTCTTTTATGCTGTGATGCTGGCTTAGGTTCTGGGAATCTGGA	1080
	H S D D V K C L C D D E G L R C W E S G	
1081	GATGACCCCTGGGTGGAAACTGCGCAAGTGGTTTCCAAAGGTGTGAGTACTTGGTCGAGAATC	1140
	D D P W V E H A K W F P R C E Y L L R I	
1141	AAAGGCCAAGAAATTTCTCAGGCCAAGCTTCAAGCTGGCTATCCTCATCTACTTGAGCAGCTA	1200
	K G Q E F V S Q V Q A G Y P H L L E Q L	
1201	TTATCTACGTCAGACTCCCCAGAGATGAGAATGCAGACGCAGCAATCGTGCATTTTGGC	1260
	L S T S D S P E D E N A D A A C V H F G	
1261	CTTGGAGAAAGATTCCGAAGATCTCTGATGATGAGCAAGCCCTCTCTGTTAAAGCAGCGTTG	1320
	P G E S S E E V V M M E T F V V H A A L	
1321	GAATGGGCTTCAGTAGGAGCTCTCTGAGACAGACGTTTCAGTGGCAGATCTCTGGCCACT	1380
	E M G F S R S C V R C T V Q W C I L A T	
1381	GTGAGAACTACAGGACCTTCAGTGAATCTTTATAGGCTTACTGCAATGCAAGAGTAG	1440
	D E N Y R T V E D L V I D L L C A E D E	
1441	ATGAGAGAGGAGGACATGAGGACAGCCGCGCTGAGTAGGAGGAGTGCAGATGATCTAGCAGTA	1500
	M R E E I M E I A A E E E S D D L A L	
1501	ATCCGGGAAGAACAAAATCTCTCTTTTCCAAACATTTGACGTGTGTGACACCAATGCTGTAT	1560
	I R H H H M V L F C H L T C V T F Y D Y	

Fig. 5 (page 2 of 3)

11/35

1561 TGGCTGCTAAGTGCAGGCGCCATCACTGACAGGAGTGCATGCTGTGAAACAGAAACCA 1520
C L L S A R A I T E Q E C N A V K Q K P -
1581 CACACCTTACAAGCAAGCACACTGATTGATACTGTGTTAGCAAAAGGAAACACTGCAGCA 1530
H T L Q A S T L I D T V L A K G N T A A -
1601 ACCTCATTTAGAAACTGCTTTGCGGAAATTTGACCTTGGCTTATACAGAGATATATTTGTC 1540
T S F R N E L R E I D P A L Y R D I F V -
1621 GACAGGACATTAGGAGTCTTTCGACAGATGACATTGCAGCTCTACCAATGGAAAGAACAG 1550
I Q D I R S L P T D D I A A L P M E E Q -
1641 TTGCGGCGCGCTGCGGAGGACAGAAATGTGTAAAGTGTGTATGAGCTGAGAGGTATGCTGC 1560
L R P L P E D E M I K V I M D R E V S I -
1661 GTGTTTCATTGCTGTGCGCATCTGCTGTGTGCAAGACTGCGCTGCTGTCTGAGGAAG 1570
V F I P C G H L V V C K D C A P S L R K -
1681 TGTCCCATCTGTAGAGGGACCATCAAGGGCACAGTGGCGACATTTCTCTGCTGAACAAGA 1580
C P I C R S T I K D T V R T F L S -
1701 CTAATGGTCCATGGCTGCAACTTCAGCCAGGAGGAAGTTCACTGTCACTCCCAAGTTCCAT 1590
TGGGAATTTGAGGCGAGCTTGATAGCAGGAGACACCGCCAAACACACAAATATAAAGAT 1600
GAAAAAGTTTTGTGTGAAGTCAAGAAATGATGAAATTAATTATATAAATTTTAATTGCT 1610
TTCTTTAAAGTGTCTATTTGTTCGCAATTCAGAAATTTTGTGTGTAACATATTTACA 1620
TACTACTTTCATGTAAGTATTTCATATATTTATATATTTAGATGTGATGAGAGAGGCTTC 1630
TGTTCCTGTTCTGTAAGGCTGCTTTATCATCTGATCAGCATATACTGCGCAACGGGCGAG 1640
GGCTAGAATGCAATGAAGCTTGAAGATGTCAGCTTAAATAAGGCTGAAAGATTTTCG 1650
AGAAACGAAAGCAAAATTTCTTTCTGTGCAATGTATACTGCTTCAGACTAATGACCTGCTTC 1660
TATCAAGGCTTTCTA 1670
1681 1674

Fig. 5 (page 3 of 3)

12/35

M-hiap2.seq

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41  ----- 120
   CACCCAAAAAAGTTAAACGTATAATGGAGAAGAGGCACAATCTTGTCAAATTGGACAAAAGAA
121 ----- 180
EQ ID NO:42  M E K S T I L S N W T K E -
181 ----- 240
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241 ----- 300
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301 ----- 360
   TACAGGTGTGAATGACAAAAGTCAAGTCTTTTGTCTGTGGCTGTATGTTGGATTAAGTGGAA
361 ----- 420
   ACAAGGGGGACAGTCTCTGTGAAAAGGACAGACAGTTCCTATCCAGCTGCAGCTTTGTACA
421 ----- 480
   TCTCTCTCTTTCAGCCAGTCTGCAGTCTCCATCTAAGAAATATGTCTCTGTGAAAAGTAG
481 ----- 540
   ATTTGCACATTCTCTCAGCTCTGGAAAGAGGTGGCAATCACTGCAACCTGTCTCTAGCCG
541 ----- 600
   TCTTAATTCTAGAGGAGTGGAGAGCTTCTCATCAAGGATGGATCCCTGCAGCTATGCCAT
601 ----- 660
   SAGTACAGAAGAGGGCCAGATTTCTTACTTACAGTATCTGGCTTTTAAGTTTCTCTGCACC
661 ----- 720
   AGCAGAGCTTGGCCAGAGCTTGGCTTTTATTACATAGGGCTTGGAGACAGGGTGGCTTGTTC
721 ----- 780
   TGGCTGTGGTGGGAAAGTGAAGCAACTGGGAAAGCAAGGATTATGCTATGTCTAGAGCACCG
781 ----- 840
   A C G G K L S N W E P K D Y A M S E H R

```

Fig. 6 (page 1 of 3)

	CAGACATTTTCTCTACTSTCCATTTCTGTGAAAAATACTTCAGAACAACACAGAGCTTTAGTAT	
751	R H F P H C P F L E N T S E T Q R F S I .	849
	ATCAAAATTTAAGTATCGCAGACACACTCTCTCTGGATTGAGGACATTTCTGTACTGGGCCACC	
841	S M L S M T T H S A R L R T F L Y W P P .	939
	TACTCTCTCTCTCTCTCACCTTGAGCAGCTTGCAAATCTCTGGATTCTATTACTGGATCGCAA	
931	S V P V Q F E L L A S A D F Y Y W D R N .	969
	TTATCATCTTCAASTCTCTTTTTCTGTCTATCTCTGCTTTCAGATCTTTGGGAACCTCGAGATGA	
961	D C V K C L C C D G G L R C W E P G D D .	1029
	CCCCCTGGATAGAACACGCGCAAAATCTCTTTCAGCTTCTGACTTTCTTATACCGATGAAGGG	
1021	F W L E H A K W F P R C E F L I R M K G .	1089
	TCAGGAGCTTTCTTGATGAGATTCAAGCTAGAGATCTCTCATTTCTCTTGAGCAGCTCTTTCTC	
1081	Q E F V D E I Q A R Y P H L L E Q L L S .	1149
	CATTTTCAGACACCTCGAGGAGAAGAAATCTCTGACCTTAGAGACACAGTCTCTGCAATTTTGG	
1141	T S D T P G E E N A D P T E T V V H F G .	1209
	CTCTGGAGAAAAGTTGCAAAAGATCTCTCTCATCATGAGCAGCCCTCTCTCTTAAAGCAGCCCTT	
1201	P G E S S K D V V M M S T P V V H A A L .	1269
	TCAAAATCTCTTTCACTAGGAGCCCTCTCTGAGACAGACCTTTCTAGCTTCAGATCTCTGGCCAC	
1261	E M G F S R S L V R C T V I R I L A T .	1329
	TGCTGAGAACTACAGGACCCCTCAATGCATTTCTCTCAGTACTTTTCAATCTCTGAAGATGA	
1321	S E N Y R T V M D I V S V L L H A E D E .	1389
	GAGAAAGATAAGAGGAGAAAGGAAAGAAATCTGAAGAGATCTCTATCAGCTGCAATTATCACT	
1381	R R E E E K E R I T E E M A S G D L S L .	1449
	GATTCGGAAAGCAATAGAAATGCGCCCTCTTTCAACATTTTCACACATCTCTCTCTCTATCTCTGA	
1441	I R K N E M A L E I I L T H V D P I G D .	1509

BNSDOCID: <WO 9708265A2 | >

14/35

TAATCTTCTTGAGGCCAGTGTAAATTACAAAACAGGAACATGATATTATTAGACAGAAAAC
1501 1550
N L L E A S V I T K Q E H D I I R Q K T -

ACAGATACCCTTACAAGCAAGAGAGCTTATTGACACCGTTTTAGTCAAGGGAAAATGCTGC
1551 1600
Q I F L Q A R E L I D T V L V K G N A A -

AGCCAACATCTTCAAAAACCTCTCTGAAGGGAAATGACTCCACCTTATGAAAACCTTATT
1601 1650
A N I F K N S L K D I D E T L Y E N L F -

TGTGGAAAAGAATATGAAGTATATTCCAAACAGGAAGACCTTTCAGGCTTGTCTATTGGAAGA
1651 1700
V E K N M K Y I P T E D V S G L S L E E -

GCAGTTGCGGAGATTACAGAAGAACTGAACCTTCCAAATGTCTATGACAGAGAGGTTTTG
1701 1750
Q L R R L Q E E R T C K V C M D R E V S -

TATTGTGTTTCATTCCCTGTGTGTCATCTAGTAGTGTGCGCAGGAATGTGCCCCCTTCTCTAAG
1751 1800
I V F I P C G H L V V C Q E C A P S L R -

GAAGTGGCCCATCTGCAGGGGGACAACTCAAGGGGACTGTGCGGACATTTCTCTCATGAGT
1801 1850
K C P I C R G T I K G T V R T F L S -

GAAGAATGGTCTGAAAGTATTGTTGGACATCAGAAGCTGTGAGAACAAGAATGAACCTAC
1851 1900
TGATTTTCAGCTTTTCAGCAGGACATTCTACTCTCTTTCAAGATTAGTAATCTTGTCTTAT
1901 1950
GAAGGTTAGCATTGTATATTTAAGCTTAGTCTGTGGAAGGGAAGTTCTATGCTTGTGAG
1951 2000
CTACAGGACTGTGTCTGTTCCAGAGCAGGAGTTGGGATGCTTGCTGTATGTCTTTCAGGA
2001 2050
CTTCTGGGATTTGGGAATTTGGGAAAGCTTTGGAATCCAGTGATGTGGAGCTCAGAAA
2051 2100
TGCTTGAACCACTGACTCTGGTACTCAGTAGATAGGGTACCTGTACTTCTTGTGCTTT
2101 2150
TCCAGTCTGGGAATAAGGAGGAATGTCTGCTGTGTAATAATTTGCTGGATGTGAGAAAT
2151 2200
AGATGAAGTGTGCTGGGTGGGGGCTGTATCAAGTGTAGTGTGTGAGGGATGTATGAG
2201 2250
GCCAAACACTGTGTAG
2251 2300
2301 2350
2351 2400
2401 2450

Fig. 6 (page 3 of 3)

16 / 35

File. 8 (page 1 of 3)

17/35

	301	BIR 3				350
cp-lap	qspECHACAG	FFYGGYGGG	KCFYCGGGG	cWepeDvPWe	CHVGFHGGG	
diap	qpsaLAAG	LYYKXGGG	KCFHCHGGG	sWqkeGGPWe	eHAKWsfKGG	
m-xiap	VnxEGLAAG	FYVGGGG	KCFHCHGGG	cWkpsGGPWe	CHAKWsfKGG	
xiap	VnxEGLAAG	FYVGGGG	KCFHCHGGG	cWkpsGGPWe	CHAKWsfKGG	
hiap1	VnxEGLAAG	FYVGGGG	KCFHCHGGG	cWkpsGGPWe	CHAKWsfKGG	
hiap2	VnxEGLAAG	FYVGGGG	KCFHCHGGG	cWkpsGGPWe	CHAKWsfKGG	
consensus	V---EGLAAG	FYY---GGG	KCF---GGG	---W---GGP---	CHAKWsfKGG	
351						
cp-lap	VnxEGKGGG	VnxVGGG	VnxVGGG	VnxVGGG	VnxVGGG	
diap	VnxEGKGGG	VnxVGGG	VnxVGGG	VnxVGGG	VnxVGGG	
m-xiap	VnxEGKGGG	VnxVGGG	VnxVGGG	VnxVGGG	VnxVGGG	
xiap	VnxEGKGGG	VnxVGGG	VnxVGGG	VnxVGGG	VnxVGGG	
hiap1	VnxEGKGGG	VnxVGGG	VnxVGGG	VnxVGGG	VnxVGGG	
hiap2	VnxEGKGGG	VnxVGGG	VnxVGGG	VnxVGGG	VnxVGGG	
consensus	V---EGKGGG	V---VGGG	V---VGGG	V---VGGG	V---VGGG	
401						
cp-lap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
diap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
m-xiap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
xiap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
hiap1	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
hiap2	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
consensus	V---VLPgg	V---VLPgg	V---VLPgg	V---VLPgg	V---VLPgg	
451						
cp-lap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
diap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
m-xiap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
xiap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
hiap1	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
hiap2	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
consensus	V---VLPgg	V---VLPgg	V---VLPgg	V---VLPgg	V---VLPgg	
501						
cp-lap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
diap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
m-xiap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
xiap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
hiap1	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
hiap2	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
consensus	V---VLPgg	V---VLPgg	V---VLPgg	V---VLPgg	V---VLPgg	
551						
cp-lap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
diap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
m-xiap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
xiap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
hiap1	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
hiap2	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
consensus	V---VLPgg	V---VLPgg	V---VLPgg	V---VLPgg	V---VLPgg	
601						
cp-lap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
diap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
m-xiap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
xiap	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
hiap1	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
hiap2	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	VnxVLPgg	
consensus	V---VLPgg	V---VLPgg	V---VLPgg	V---VLPgg	V---VLPgg	

Fig. 8 (page 2 of 3)

18/35

	551	Ring Zinc Finger		560
cp-lapEkepq	veDskLCKIC	yveEolVcSV	
diap	snisklcccl	ekmsvscpg	nlslEenRq	LxDarLCKVC
m-xiap	elstEQLAR	LqSEKLSKID	MDenLIIVFI
xiap	elstEQLAR	LqSEKLCNIC	MDenLIIVFI
hiap1	lyenlfvqqe	mkypscdvs	clpveQLAR	LpSEKCKVC
hiap2	lyenlfvckn	mkypscdvs	elstEQLAR	LqSEKCKVC
consensus	-----	---S---QLAR	L-EE-LCK-C	MD-SV---VF-
	501		635	
cp-lap	PCGRLVAcAN	CALEVCKCFM	CRKIVervik	VYFS.
diap	PCGRLatCnq	CApSVanCFM	CRacIKqfv	LFLS.
m-xiap	PCGRLatCkq	CAeavCKCFM	CyevIIdnq	LFMS.
xiap	PCGRLVtCkq	CAeavCKCFM	CyevIIdkq	LFMS.
hiap1	PCGRLVvCkd	CApSLKCP	CRacIKqfv	LFLS.
hiap2	PCGRLVvCde	CApSLKCP	CRacIKqfv	LFLS.
consensus	PCGRLV-C--	CA-SV-KCFM	CR--I-----	-FLS-

Fig. 8 (page 3 of 3)

19/35

Alignment of ZNF (Ring Zinc Finger) Domains

Helovirus	
Co_30	Cyclia gemmata
Co_30	Orgyia pseudotsugata
nan	
slac	MAP on X chromosome
slac1 slac2	two different human MAP genes
use	
m-xlac	mouse homologue of human slac gene
lect	
slac	Drosophila MAP gene, not clearly a homologue of slac or slac1

is an consensus. The consensus line represents amino acids or very similar amino acids which are present in 6 of the 10 B22 sequences at each position. Capitalized residues are those that are in the consensus sequence.

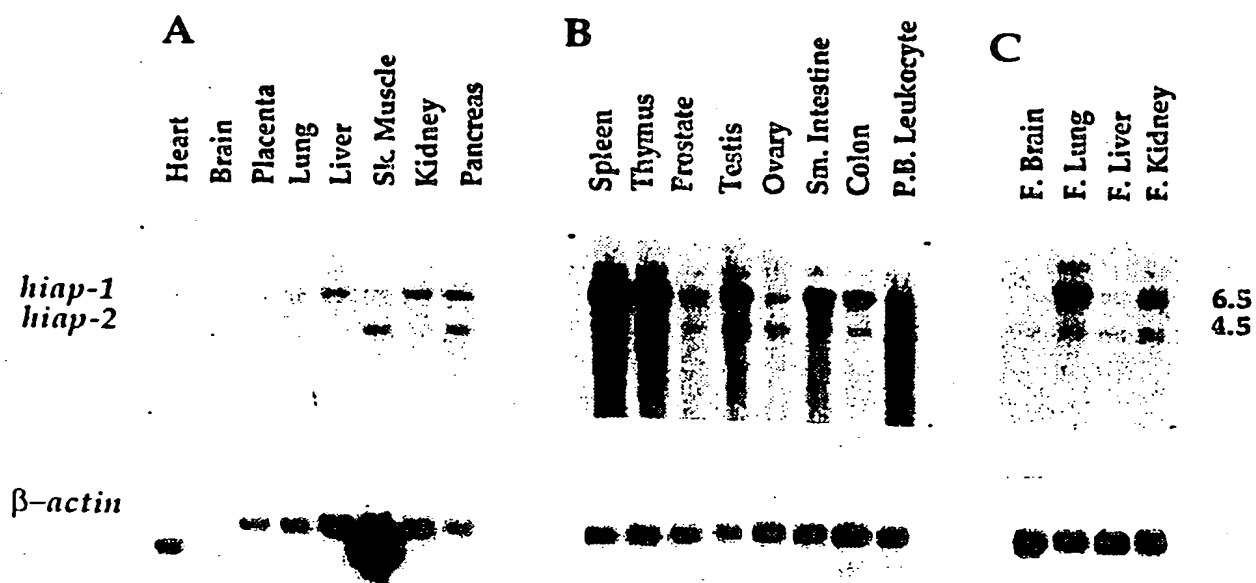
46

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SEQ ID NO:34	100	24122112222	10000000000	11111111111	11111111111	11111111111	11111111111
SEQ ID NO:35	10	24122112222	10000000000	11111111111	11111111111	11111111111	11111111111
SEQ ID NO:36	10	24122112222	10000000000	11111111111	11111111111	11111111111	11111111111
SEQ ID NO:37	21ap	24122112222	10000000000	11111111111	11111111111	11111111111	11111111111
SEQ ID NO:38	21ap	24122112222	10000000000	11111111111	11111111111	11111111111	11111111111

consensus

Fig. 9

20/35

FIG. 10

SUBSTITUTE SHEET (RULE 26)

21/35

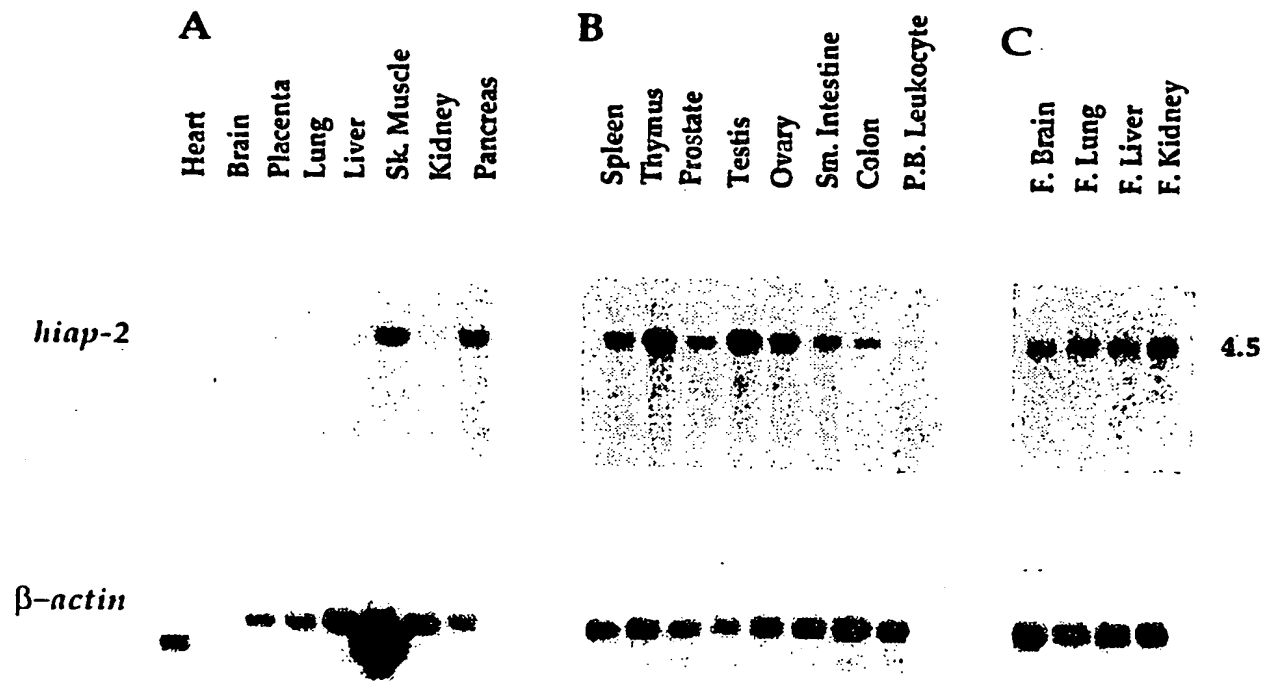


FIG. 11

SUBSTITUTE SHEET (RULE 26)

22/35

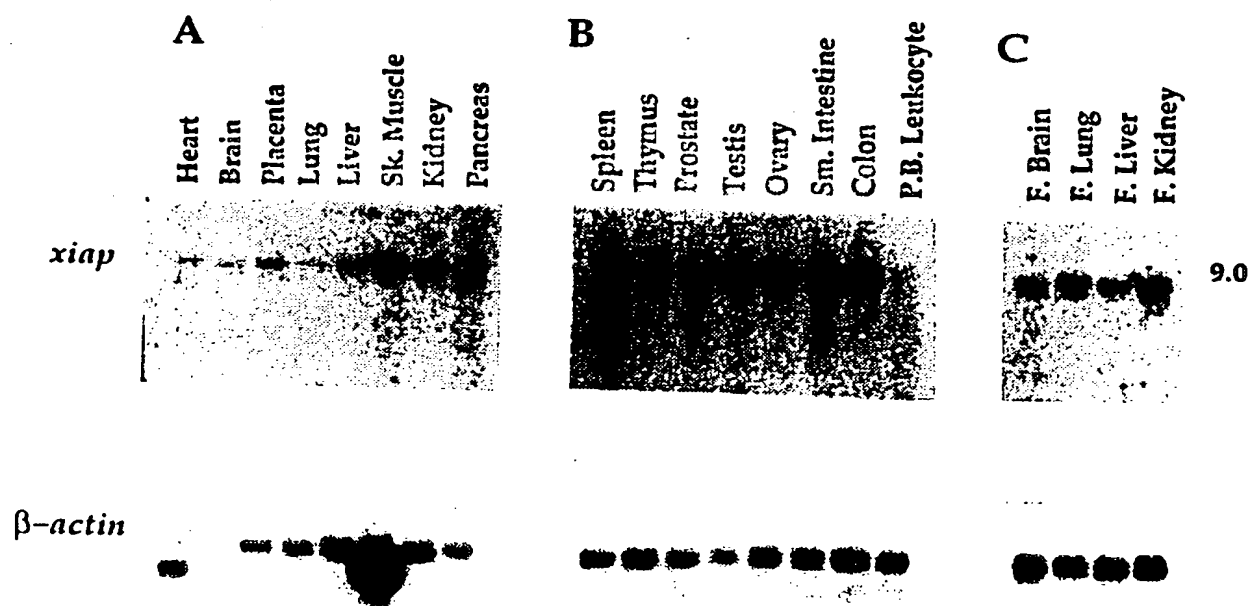
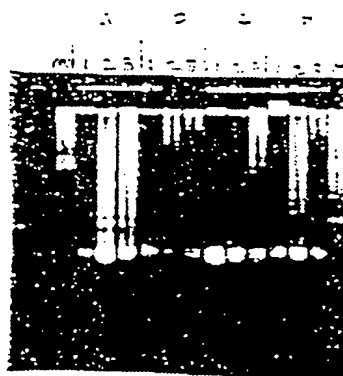


FIG. 12

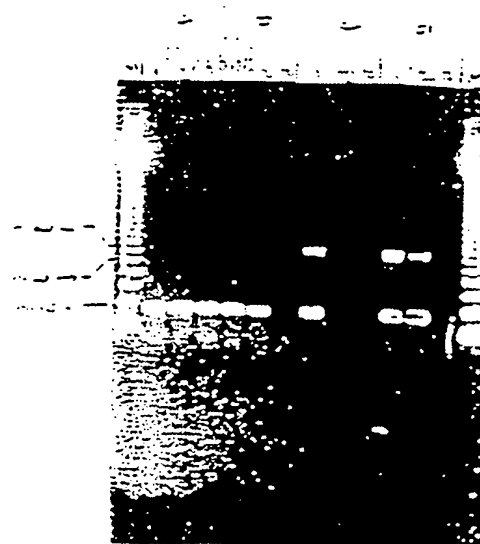
SUBSTITUTE SHEET (RULE 26)

23/35

13A



13B



24/35

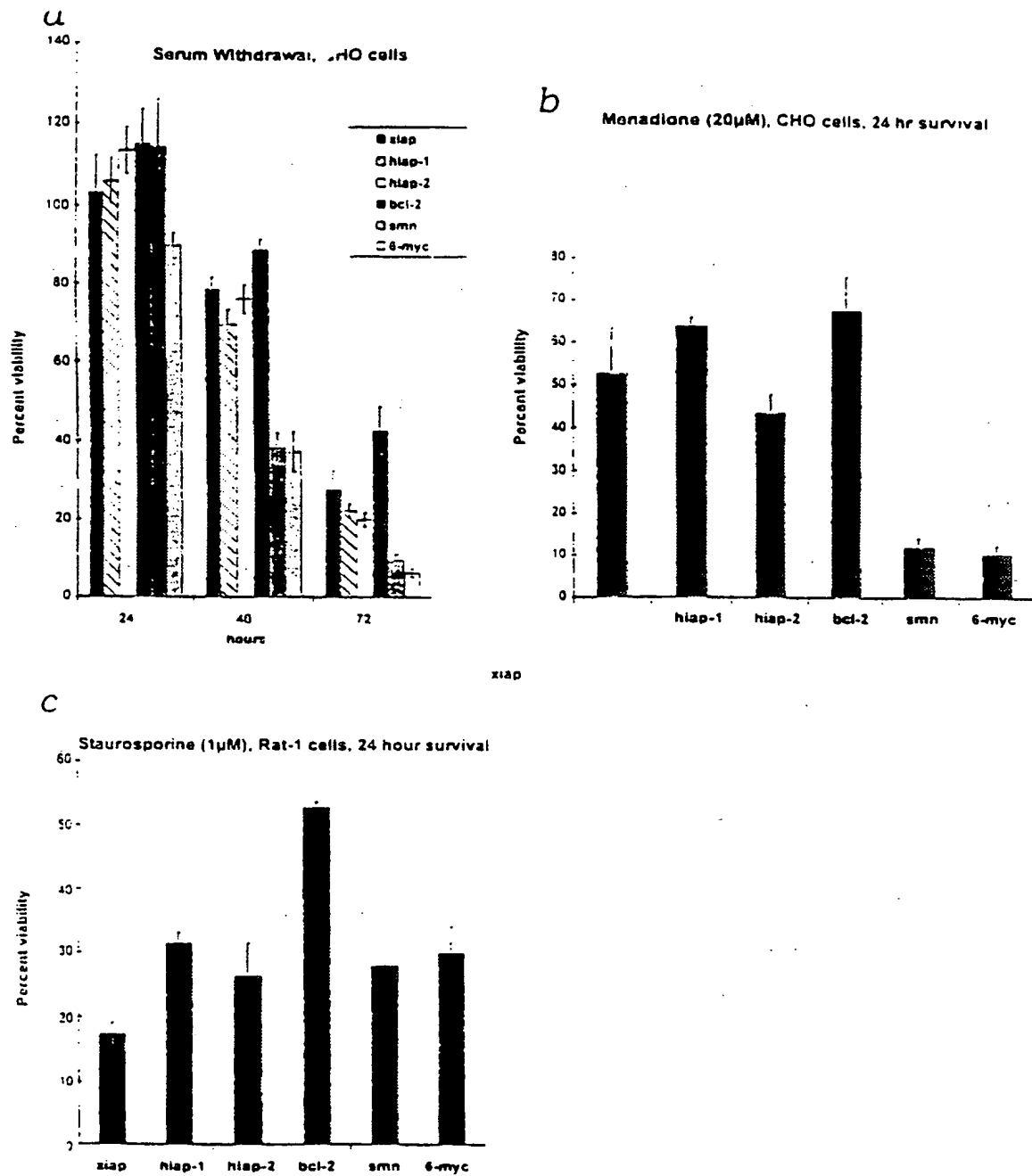
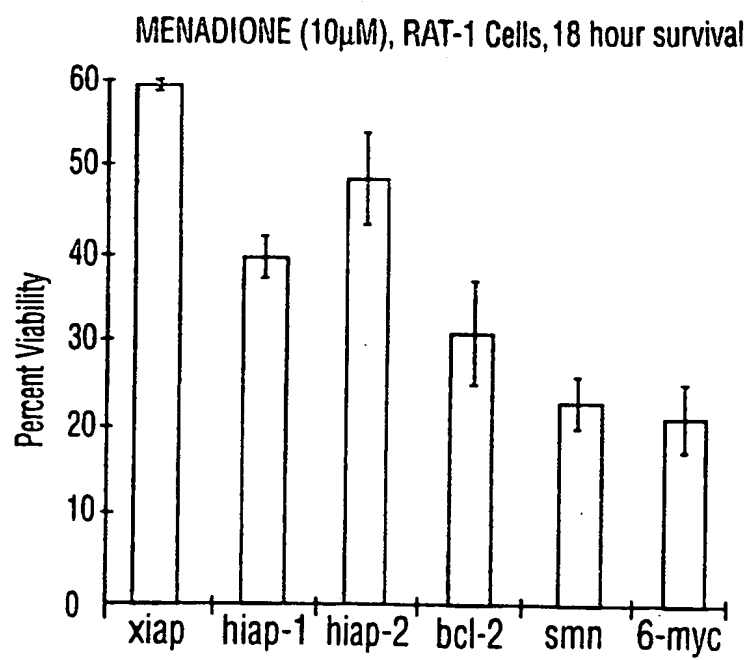


Fig. 14A - C

24-1/35

**FIG.14D**

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25/35

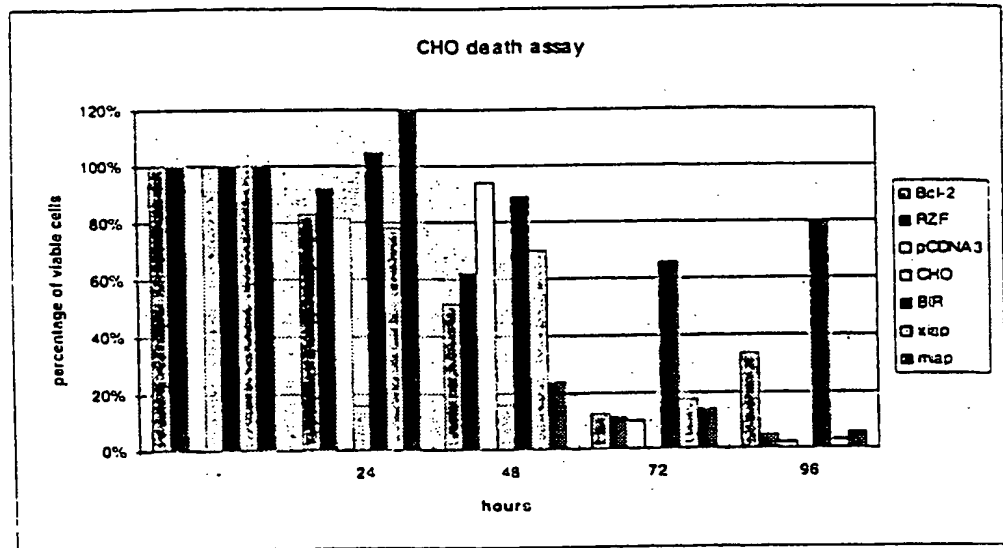


Figure 15A

26/35

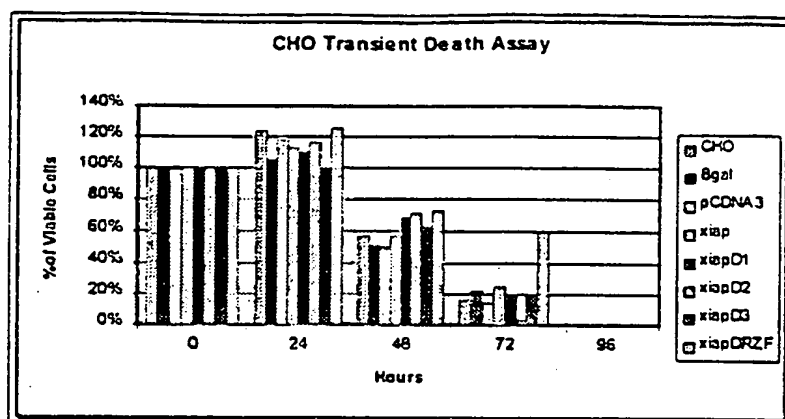


Figure 15B

27 / 35

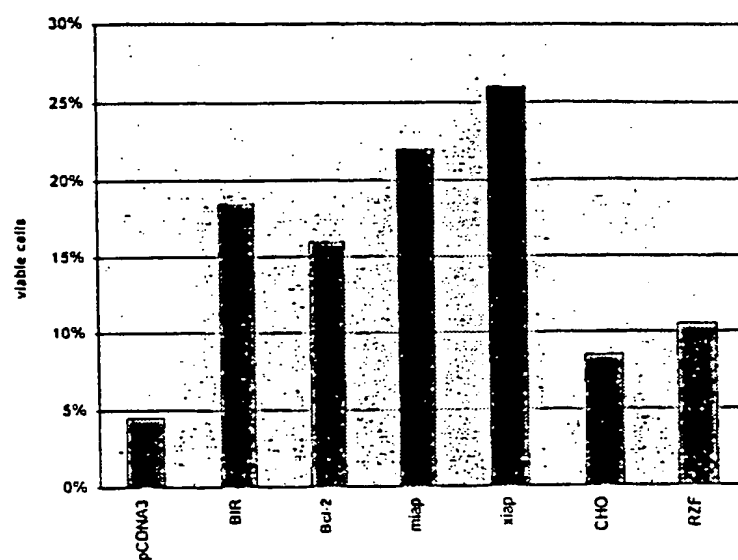


Figure 16A

28/35

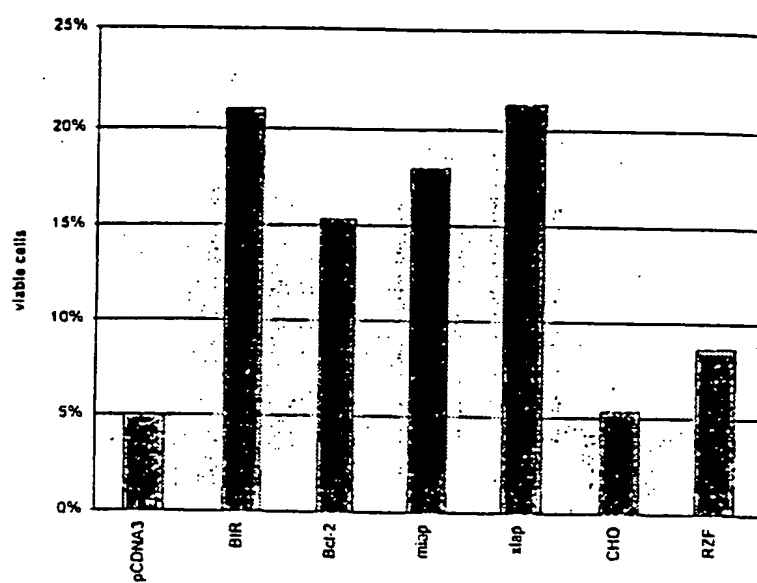


Figure 16B

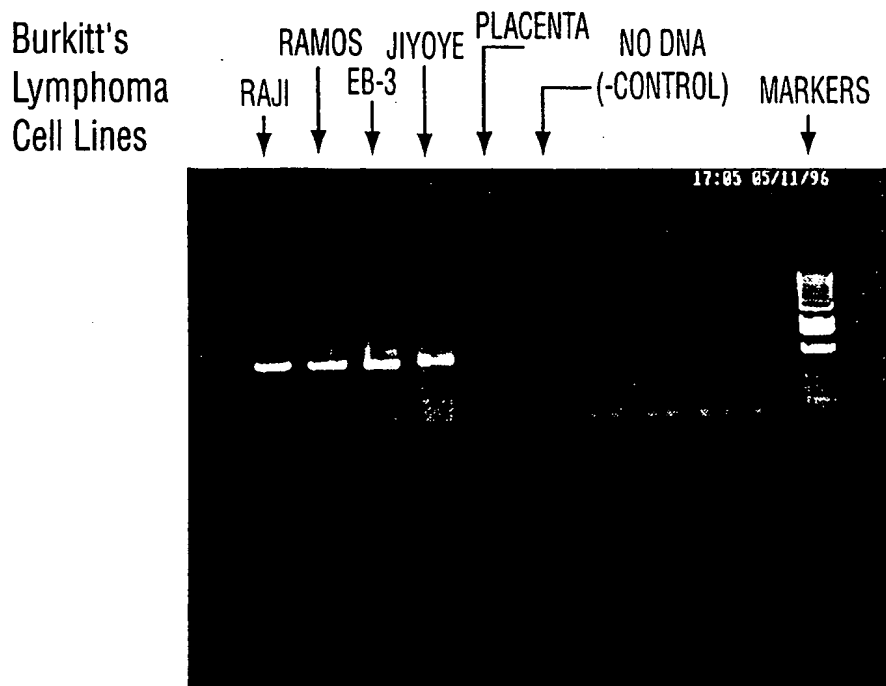


FIG. 17

30/35

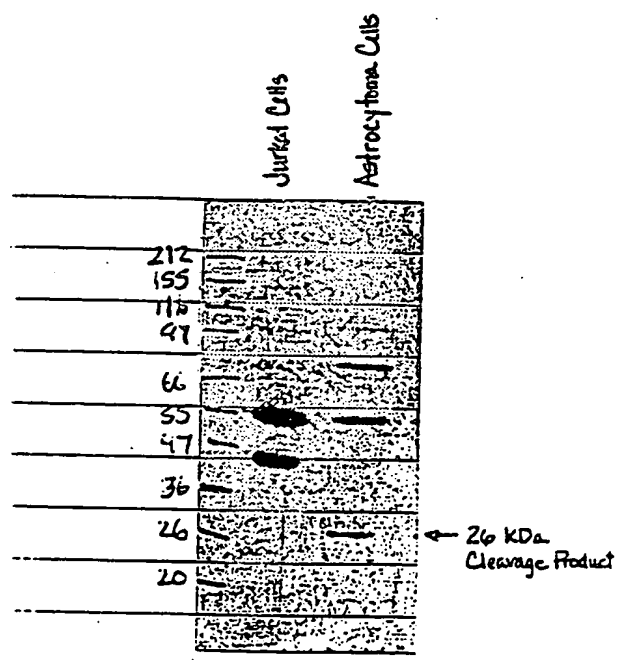


Figure 18

31/35

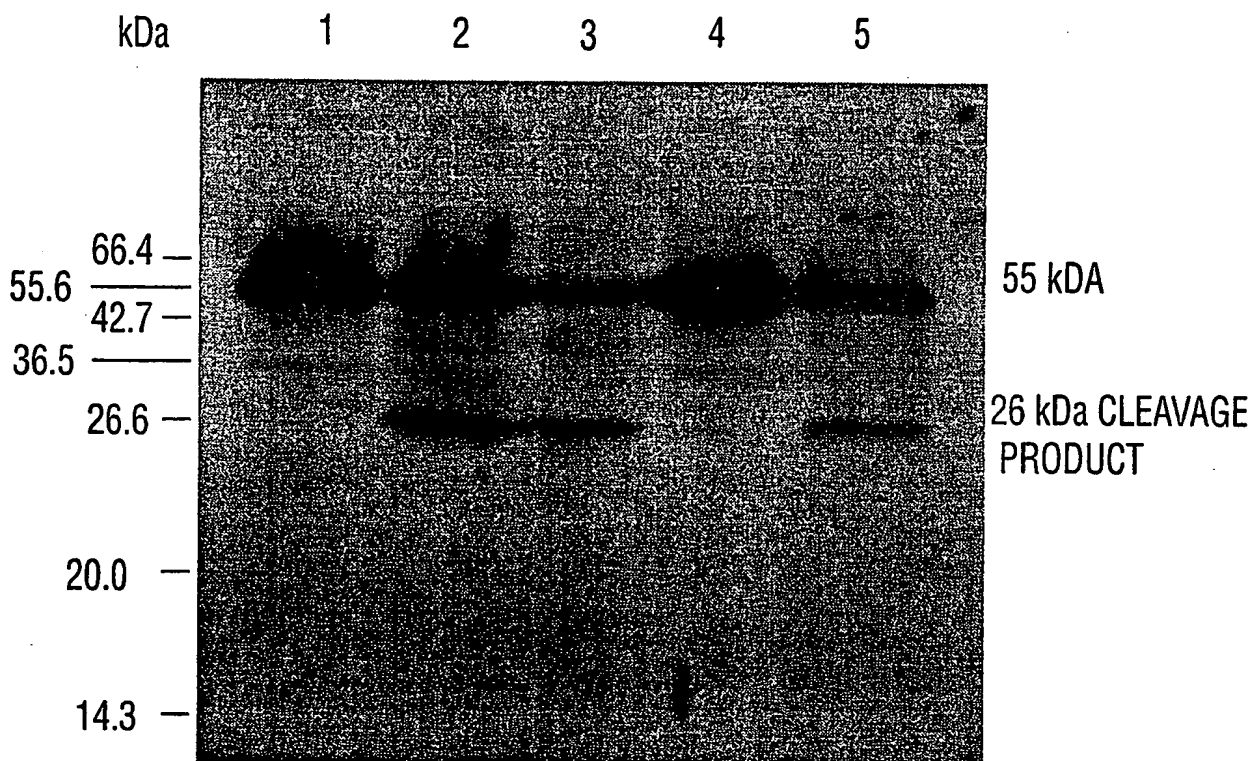


FIG.19

32 / 35

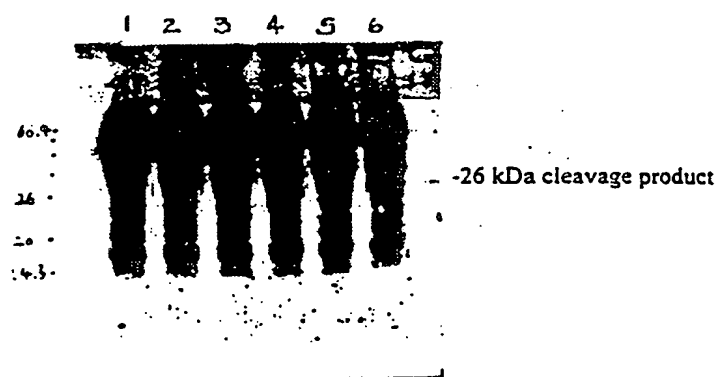


Figure 20

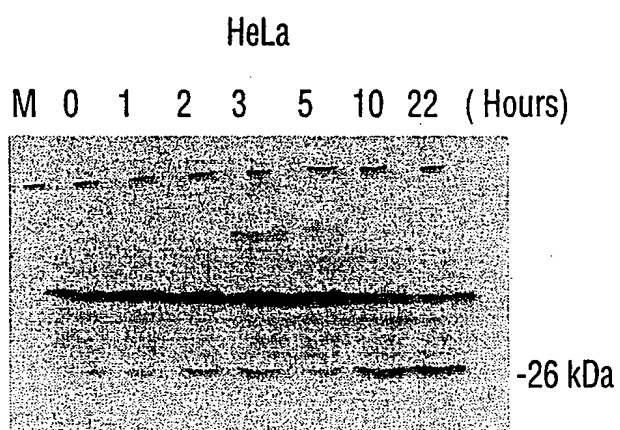


FIG. 21A

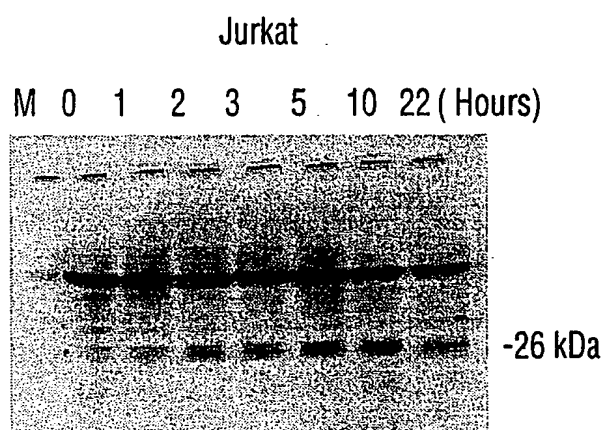


FIG. 21B

34/35

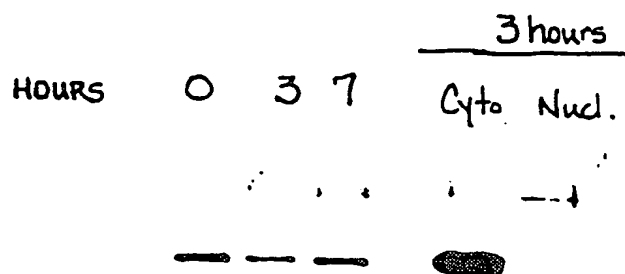


Figure 22A

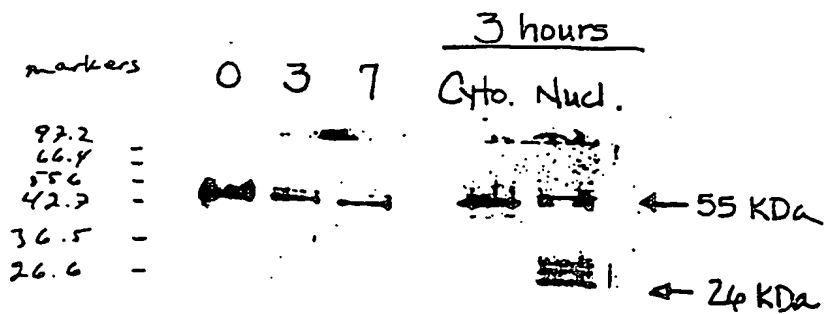


Figure 22B

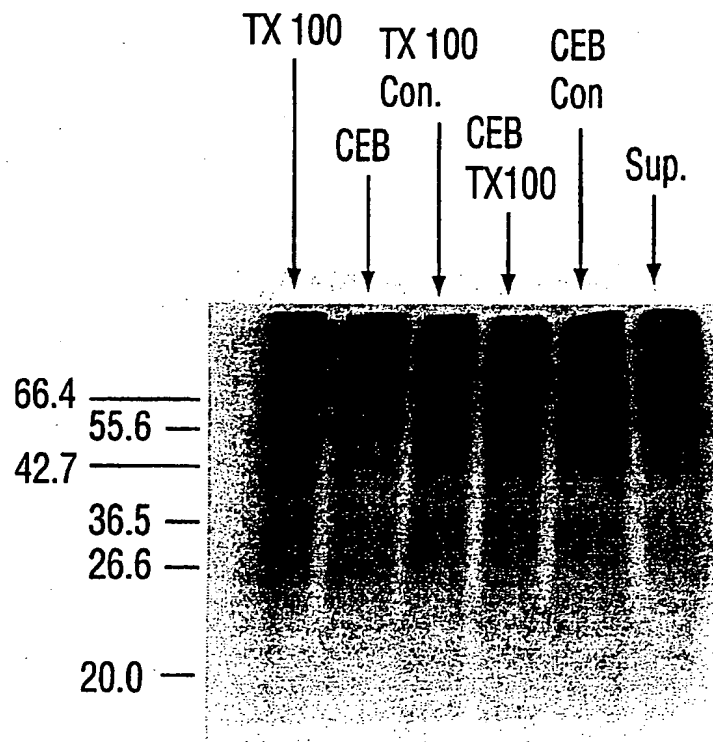


FIG. 23

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76962-1

9 January 1997

International Searching Authority
European Patent Office
P.B. 5818 Patenlaan 2
NL-2280 HV Rijswijk
NETHERLANDS

Dear Sir:

Re: PCT Patent Application
Serial No. PCT/IB96/01022
University of Ottawa
(Robert G. Korneluk, et al)

We refer to the Invitation to Correct Defects dated October 3, 1996, copy attached, and in particular to the additional observations referred to therein. The Receiving Office of the International Bureau has objected to the poor quality of printing of the drawings, and has directed applicant to Rule 91 relating to the rectification of obvious errors. It is our belief that the authorized International Searching Authority to whom our request for rectification of obvious errors should be directed is ISA/EP.

This letter is filed pursuant to Rule 91.1(e)(11) to request authorization by the International Searching Authority of rectification of obvious errors in the drawings. Such a request should have been filed within 17 months of the priority date.

However, we have only recently been appointed agent with respect to this application, and even more recently become aware of the Invitation to Correct Defects and the requirements therein relating to drawings. Since becoming aware of the situation, we have been diligently pursuing the matter with applicant's US patent attorney and applicant's licensee. The drawings originally filed were apparently photocopies of drawings prepared for the priority application, thus the relatively poor quality. It was necessary to locate the original versions of each of the drawings, some of which were with the US patent attorney and some of which were with the inventors. Fresh formal drawings meeting the PCT requirements then had to be prepared from these originals. All of these events were further delayed by the holiday season.

We have now completed preparation of amended drawings. These have been filed in the International Bureau Receiving Office, in response to the outstanding Invitation to Correct Defects, a response to which is due January 10, 1997. We enclose copies of our letter to the International Bureau together with the amended sheets proposed for replacement.

We understand that a request for rectification of an obvious error may be entertained under Rule 91.1 so long as technical preparations for international publication have not yet been completed. We accordingly request that the International Searching Authority consider this matter before completing technical preparations for publication, and grant the requested request for rectification.

The "errors" in the drawings appear to reside in the fact that their quality is such that in at least some respects they are not clearly readable. Although the quality of the drawings originally filed admittedly is poor, a comparison of the drawings originally filed with the enclosed amended drawings reveals that the content is obviously the same. Further with respect to those of the Figures which refer to sequence listings, the content of the amended drawings can be verified by a comparison with the print copy of the sequence listing which accompanied the application when filed and which forms part of the description. For these reasons, applicant respectfully submits that the "errors" in the drawings are obvious in that anyone would immediately realize that nothing else could have been intended other than what is offered as rectification.

In all of thes circumstances, we request that the International Searching Authority authorize rectification of obvious errors in the drawings, and acceptance of the replacem nt sheets submitted herewith.

Yours very truly,

SMART & BIGGAR

(Mrs.) Joy D. Morrow

HUMAN xiap

SEQ ID NO:3

1 gaaaagggtggacaagtccctaatttcaagagaagatgacttttaacagttttgaaggatctt
60

SEQ ID NO:4 a

M T F N S F E G S -

61 aaaacttggtacctgcagacatcaataaggaagaattttagaagaggtttaataga
120

a K T C V P A D I N K E E F V E E F N R -

121 ttaaaaacttttgctaattttccaagtggtagtcctgtttcagcatcaacactggcacga
180

a L K T F A N F P S G S P V S A S T L A R -

181 gcagggttttttatactggtgaaggagataccgtgcggtgctttagttgtcatgcagct
240

a A G F L Y T G E G D T V R C F S C H A A -

241 gtagatagatggcaatatggagactcagcagttggaagacacaggaagatcccccaat
300

a V D R W Q Y G D S A V G R H R K V S P N -

301 tgcagatttatcaacgggttttattcttgaataatagtgccacgcagttctacaaattctggt
360

a C R F I N G F Y L E N S A T Q S T N S G -

FIG. 1 (PAGE 1 OF 7)

2/61

HUMAN xiap

```

361  atccagaatggtcagtacaaagtgaataactatctgggaagcagagatcatttgcctta
-----+-----+-----+-----+-----+
a    I Q N G Q Y K V E N Y L G S R D H F A L -
-----+-----+-----+-----+-----+
421  gacaggccatctgagacacatgcagactatcttttgagaactgggcaggtttagatatata
-----+-----+-----+-----+-----+
a    D R P S E T H A D Y L L R T G Q V V D I -
-----+-----+-----+-----+-----+
481  tcagacaccatatacccgaggaaacctgccatgtattgtgaagaagctagattaaagtcc
-----+-----+-----+-----+-----+
a    S D T I Y P R N P A M Y C E E A R L K S -
-----+-----+-----+-----+-----+
541  ttccagaactggccagactatgctcacctaaaccccaagagagttagcaagtgtggactc
-----+-----+-----+-----+-----+
a    F Q N W P D Y A H L T P R E L A S A G L -
-----+-----+-----+-----+-----+
601  tactacacagggtattggtgaccaagtgcagtgttttctgtgtggtggaactgaaaaat
-----+-----+-----+-----+-----+
a    Y Y T G I G D Q V Q C F C C G G K L K N -
-----+-----+-----+-----+-----+
661  tgggaaccttgtgatcgtgcctggtcagaacacagggcacactttccctaattgcttcttt
-----+-----+-----+-----+-----+
a    W E P C D R A W S E H R R R H F P N C F F -
-----+-----+-----+-----+-----+
720

```

FIG. 1 (PAGE 2 OF 7)

3/61

HUMAN xiap

```

gttttggcggaatcttaatatcgaagtgaatctgatgctgtgagttctgataggaat 780
-----+-----+-----+-----+-----+
a      V L G R N L N I R S E S D A V S S D R N -

ttcccaaatccaacaaatcttccaagaaatcccatccatggcagattatgaagcacggatc 840
-----+-----+-----+-----+-----+
a      F P N S T N L P R N P S M A D Y E A R I -

tttacttttgggacatggatatactcagtttaacaaggagcagcttgcaagagctggattt 900
-----+-----+-----+-----+-----+
a      F T F G T W I Y S V N K E Q L A R A G F -

tatgcttttaggtgaagggtgataaaagtaaagtgcctttcactgttgaggagggttaactgat 960
-----+-----+-----+-----+-----+
a      Y A L G E G D K V K C F H C G G G L T D -

tggaagcccagtgaaagacccttgggaacaacatgctaaatgggtatccagggtgcaaatat 1020
-----+-----+-----+-----+-----+
a      W K P S E D P W E Q H A K W Y P G C K Y -

ctgttagaacagaagggaagaatatataaacaatatctcatttaactcattcacttgag 1080
-----+-----+-----+-----+-----+
a      L L E Q K G Q E Y I N N I H L T H S L E -

```

FIG. 1 (PAGE 3 OF 7)

4/61

HUMAN xiap

```

1081      gagtgcctggtaagaactactgagaaaaacaccatcactaactagaagaattgatatacc 1140
      -----+-----+-----+-----+-----+-----+-----+-----+
a      E C L V R T T E K T P S L T R R I D D T -

1141      atcttccaaatcctatggtacaagaagctatacgaaatggggttcagtttcaaggacatt 1200
      -----+-----+-----+-----+-----+-----+-----+-----+
a      I F Q N P M V Q E A I R M G F S F K D I -

1201      aagaaaaataatggaggagaaaaaattcagatatctctgggagcaactataaatacacttgaggtt 1260
      -----+-----+-----+-----+-----+-----+-----+-----+
a      K K I M E E K I Q I S G S N Y K S L E V -

1261      ctggttgcagatctagtgaatgctcagaagaagacagtatgcaagatgagtcgaagtcagact 1320
      -----+-----+-----+-----+-----+-----+-----+-----+
a      L V A D L V N A Q K D S M Q D E S S Q T -

1321      tcattacagaaagagatttagtactgaagagcagctaaggcgcttgcaagaggagaagctt 1380
      -----+-----+-----+-----+-----+-----+-----+-----+
a      S L Q K E I S T E E Q L R R L Q E E K L -

1381      tgcaaaaatctgtatggatagaaatattgctatcggttttcttcttctgtggacatctagtc 1440
      -----+-----+-----+-----+-----+-----+-----+-----+

```

FIG. 1 (PAGE 4 OF 7)

5/61

HUMAN xiap

```

a      C K I C M D R N I A I V F V P C G H L V -
      acttgtaaacaaatgctgaagcagttgacaagtggtcccatgtgctacacagtcattact
1441 -----+-----+-----+-----+-----+ 1500
      T C K Q C A E A V D K C P M C Y T V I T -
      ttcaagcaaaaaatttttatgtctttaaactctatatagtaggcattgtttgttct
1501 -----+-----+-----+-----+-----+ 1560
      F K Q K I F M S *
      tattaccctgattgaatgtgtgatgtgaactgactttaagtaatcaggattgaattcccat
1561 -----+-----+-----+-----+-----+ 1620
      tagcatttgctaccaagtaggaaaaaaatgtacatggcagtggttttagttggcaatata
1621 -----+-----+-----+-----+-----+ 1680
      atctttgaatttcttgatttttccagggtattagctgtattatccatttttttactgtta
1681 -----+-----+-----+-----+-----+ 1740
      ttttaattgaaaccatagactaagaataagaagcatcatataactgaacacaatgtgt
1741 -----+-----+-----+-----+-----+ 1800

```

FIG. 1 (PAGE 5 OF 7)

6/61

FIG. 1 (PAGE 6 OF 7)

HUMAN xiap

2161 nagatatgttaagtgtaaaatgcaagtggcnnacactatgtatagtctgagccagatca 2220

८

2221 aagtatgtatgttnttaatatgcataagaacnanagatttgaaagatatcaccaactg 2280

४

2281 ttaaatgtggtctctcttcgaggagggttgaggagggggcccccagaggggtttta
-----+-----+-----+-----+-----+ 2340

८७

2341 naggggccttttcacttttcnacttttttcattttgttcgttcgnatttttataagtat 2400

॥

2401 gnanacccnaagggttttatgnaactaacatcagtaacctaacccccgtgactacct
-----+-----+-----+-----+-----+-----+ 2460

॥

gtnctcttcctaggaggctgnttgtttcccaaccaccaccttccctctgaacaaatgc
-----+-----+-----+-----+-----+-----+ 2520

ॐ

2521 ctgagtgctggggcactctn
-----+-----+ 2540

८

FIG. 1 (PAGE 7 OF 7)

HUMAN hiap-1

SEQ ID NO:5

1 TCCTTGAGATGATCAGTATAGGATTTAGGATCTCCATGTTGGAACCTCTAAATGCATAGA
60

C

61 AATGGAAATAATGGAAATTTTCATTTTGGCTTTCAGCCCTAGTATTAAACTGATAAAA
120

C

121 GCAAAGCCATGCACAAAACCTACCTCCCTAGAGAAAGGCTAGTCCCTTTTCTTCCCCATTC
180

C

181 ATTTTCATTATGAACATAGTAGAAAACAGCATATTCTTATCAAAATTGATGAAAAGCGCCA
240

SEQ ID NO:6

241 ACACGTTTGAACTGAAATACGACTTGTCTCATGTGAACTGTACCGAATGTCTACGTATTCCA
300

C

301 T F E L K Y D L S C E L Y R M S T Y S T
CTTTCCTGCTGGGTTCCCTGTCTCAGAAAGGAGTCTTGCTCGTGGTTTCTATTACA
360

C

F P A G V P V S E R S L A R A G F Y Y T

FIG. 2 (PAGE 1 OF 8)

8/61

9/61

HUMAN hiap-1

```

361 CTGGTGTGAATGACAAGGTCAAATGCTTCTGTGTGGCCTGATGCTGGATAACTGGAAAA
      G V N D K V K C C F C C G L M L D N W K R -
421 GAGGAGACAGTCCTACTGAAAAGCATATAAAGTTGTATCCTAGCTGCAGATTTCGTTTCAGA
      G D S P T E K H K K L Y P S C R F V Q S -
481 GTCTAAATTCCGTTAAACAACCTTGGAAAGCTACCTCTCAGCCTACTTTTCTTCTTCAGTAA
      L N S V N N L E A T S Q P T F P S S V T -
541 CACATTCACACACTCATTACTTCGGGTACAGAAAACAGTGGATATTTCCGGTGGCTCTT
      H S T H S L L P G T E N S G Y F R G S Y -
601 ATTCAAACCTCTCCCATCAAATCCCTGTAAACTCCAGAGCAAATCAAGAAATTTCTGCCTTGA
      S N S P S N P V N S R A N Q E F S A L M -
661 TGAGAAAGTTCCTACCCCTGTCCCAATGAATAACGAAAATGCCAGATTACTTACTTTTCAGA
      R S S Y P C P M N N E N A R L L T F Q T -

```

FIG. 2 (PAGE 2 OF 8)

10/61

HUMAN hiap-1

```

721  CATGGCCATTGACTTTTCTGTGCGCCAACAGATCTGGCACGAGCAGGCTTTTACTACATAG 780
      W P L T F L S P T D L A R A G F Y Y I G -
      C
781  GACCTGGAGACAGAGTGGCTTGCTTTGCCCTGTGGTGGAAAAATTGAGCAATTGGGAACCGA 840
      P G D R V A C F A C G G K L S N W E P K -
      C
841  AGGATAATGCTATGTCAGAACACCTGAGACATTTTCCCAAATGCCCATTTATAGAAAATC 900
      D N A M S E H L R H F P K C P F I E N Q -
      C
901  AGCTTCAAGACACTTCAAGATACACAGTTTCTAATCTGAGCATGCAGACACATGCAGCCCC 960
      L Q D T S R Y T V S N L S M Q T H A A R -
      C
961  GCTTTAAACATCTTTAACTGGCCCTCTAGTGTCTAGTTAATCCTGAGCAGCTTGCAA 1020
      F K T F F N W P S S V L V N P E Q L A S -
      C
1021 GTGCGGGTTTTTATTATGTGGTAACAGTGATGATGTCAAATGCTTTTGTCTGTGATGGTG 1080
      A G F Y Y V G N S D D V K C C F C C D G G -
      C

```

FIG. 2 (PAGE 3 OF 8)

HUMAN hiap-1

11/61

```

1081  GACTCAGGTGTTGGGAATCTGGAGATGATCCATGGGTTCAACATGCCAAGTGTTCCAA 1140
      L R C W E S G D D P W V Q H A K W F P R -

1141  GGTGTGAGTACTTGATAAGAAATTAAGGACAGGAGTTCATCCGTCAAGTTCAAGCCAGTT 1200
      C E Y L I R I K G Q E F I R Q V Q A S Y -

1201  ACCCTCATCTACTTGAACAGCTGCTATCCACATCAGACAGCCCGAGAGATGAAATGCAG 1260
      P H L L E Q L L S T S D S P G D E N A E -

1261  AGTCATCAATTATCCATTGGAACCTGGAGAAGACCATTTCAGAAGATGCAATCATGATGA 1320
      S S I I H L E P G E D H S E D A I M M N -

1321  ATACTCCTGTGATTAATGCTGCCGTGGAATGGGCTTAGTAGAAGCCTGGTAAACAGA 1380
      T P V I N A A V E M G F S R S L V K Q T -

1381  CAGTTCAGAGAAAATCCTAGCAACTGGAGAGAATTATAGACTAGTCAATGATCTTGTGT 1440
      V Q R K I L A T G E N Y R L V N D L V L -

```

FIG. 2 (PAGE 4 OF 8)

12/61

HUMAN hiap-1

```

1441 TAGACTTACTCAATGCAGAAGATGAAATAAGGGAAGAGAGAGAGAAAGAGCAACTGAGG
-----+-----+-----+-----+-----+-----+-----+
      D L L N A E D E I R E E E R A T E E -
      C

1501 AAAAGAATCAAATGATTATTATTAATCCGGAAGATAGAAATGGCACTTTTCAACATT
-----+-----+-----+-----+-----+-----+-----+
      K E S N D L L L I R K N R M A L F Q H L -
      C

1561 TGACTTGTGTAATTCCTGGATAGTCTACTAAGTCCGGAATTATTATGAACAAG
-----+-----+-----+-----+-----+-----+-----+
      T C V I P I L D S L L T A G I I N E Q E -
      C

1621 AACATGATGTTATAACAGAAGACACAGACGCTCTTACAAGCAAGAGAACTGATTGATA
-----+-----+-----+-----+-----+-----+-----+
      H D V I K Q K T Q T S L Q A R E L I D T -
      C

1681 CGATTTTAGTAAAGGAAATATTGCAGCCACTGTATTCAGAAACTCTCTGCAAGAAGCTG
-----+-----+-----+-----+-----+-----+-----+
      I L V K G N I A A T V F R N S L Q E A E -
      C

1741 AAGCTGTGTATATGAGCATTATTGTGCAACAGGACATAAAATATATCCACAGAAAG
-----+-----+-----+-----+-----+-----+-----+
      A V L Y E H L F V Q Q D I K Y I P T E D -
      C

```

FIG. 2 (PAGE 5 OF 8)

13/61

HUMAN hiap-1

1801	ATGTTTCAGATCTACCAAGTGGAAACAATTCGGAGACTACCAGAAGAAACAATGTA	1860
C	V S D L P V E E Q L R R L P E E R T C K -	
1861	AAGTGTGTATGGACAAAAGAGTGTCCATAGTGTTCCTTGTGGTCATCTAGTAGTAT	1920
C	V C M D K E V S I V F I P C G H L V V C -	
1921	GCAAAGATTGTGCTCCTTCTTTAAGAAAGTGTCCCTATTGTAGGAGTACAATCAAGGGTA	1980
C	K D C A P S L R K C P I C R S T I K G T -	
1981	CAGTTCGTACATTTCTTTCATGAAGAAGAACCAACATCGTCTAAACTTTAGAATAAT	2040
C	V R T F L S *	
2041	TTATTAAATGATTATAACTTTAACTTTTATCCCTAATTGGTTTCCTTAAATTTTATT	2100
C		
2101	TATTACAACCTCAAAAAACATTTGTTGTGAACATATTTATATATGTATCTAAACCATA	2160
C		

FIG. 2 (PAGE 6 OF 8)

15/61

HUMAN hiap-1

2521 CAGTGTCCCTATACATCGAAGGTGTGCATATATGTTGAATCACAATTTAGGGACATGGTGT
-----+-----+-----+-----+-----+-----+-----+ 2580

C

2581 TTTTATAAAGAAATCTGTGAGXAAAAATTTAATAAGCAACCXAAATTACTCTTAAAAAA
-----+-----+-----+-----+-----+-----+-----+ 2640

C

2641 AAAAAAAAAAAAACTCGAGGGCCCGTACCAAT
-----+-----+-----+-----+-----+-----+ 2676

C

FIG. 2 (PAGE 8 OF 8)

HUMAN hiap-2

SEQ ID NO:7

1 TTAGGTTACCTGAAAGAGTTACTACAACCCCAAAGAGTTGTGTTCTAAGTAGTATCTTGG
60

a

61 TAATTCAGAGAGATACTCATCCTACCTGAATATAAACTGAGATAAAATCCAGTAAAGAAAG
120

a

121 TGTAGTAAATTCTACATAAGAGTCTATCATTTGATTCTTTTGTGGTGAATCTTAGTT
180

a

181 CATGTGAAGAAATTTCATGTGAATGTTTGTAGCTATCAAAACAGTACTGTACCTACTCATG
240

a

241 CACAAAGTGCCTCCCAAAGACTTTTCCAGGTCCCTCGTATCAAAACATTAAGAGTATA
300

SEQ ID NO:8 a

H K T A S Q R L F P G P S Y Q N I K S I
ATGGAAGATAGCACGATCTTGTGATGATGGACAAACAGCAACAAACAAAAATGAAGTAT
301

a

M E D S T I L S D W T N S N K Q K M K Y

FIG. 3 (PAGE 1 OF 7)

16/61

17/61

HUMAN hiap-2

```

361  GACTTTCCCTGTGAACCTCTACAGAATGTCTACATATTCAACTTCCCCCGGGGTGCCT
      D F S C E L Y R M S T Y S T F P A G V P -
421  GTCTCAGAAAGGAGTCTTGCTCGTGGTGTATTTATTTACTGGTGAATGACAAGGTC
      V S E R S L A R A G F Y Y T G V N D K V -
481  AAATGCTTCTGTGGCCCTGATGCTGGATAAAGTGAAGTGAAGACAGTCTATTCAA
      K C F C C G L M L D N W K L G D S P I Q -
541  AAGCATAAACAGCTATATCCCTAGCTGTAGCTTTATTCAGAAATCTGGTTTCAGCTAGTCTG
      K H K Q L Y P S C S F I Q N L V S A S L -
601  GGATCCACCTCTAAGAAATACGTCTCCAATGAGAAACAGTTTTCACATTCATATCTCCC
      G S T S K N T S P M R N S F A H S L S P -
661  ACCTTGGAACATAGTAGCTTGTTCAGTGGTCTTACTCCAGCCTTCCTCCAAACCCCTCTT
      T L E H S S L F S G S Y S S L P P N P L -

```

FIG. 3 (PAGE 2 OF 7)

HUMAN hiap-2

18/61

721 AATTCTAGAGCAGTTGAAGACATCTCTTCATCGAGGACTAACCCCTACAGTTATGCAATG 780
a N S R A V E D I S S S R T N P Y S Y A M -
781 AGTACTGAAGAAGCCAGATTCTTACCTACCATAATGTGGCCATTAACTTTTGTCCACCA 840
a S T E E A R F L T Y H M W P L T F L S P -
841 TCAGAAATTGGCAAGAGCTGGTTTTATTATATAGGACCTGGAGATAGGGTAGCCTGCTTT 900
a S E L A R A G F Y Y I G P G D R V A C F -
901 GCCTGTGGTGGAGCTCAGTAACTGGGAACCAAGGATGATGCTATGTCAGAACACCGG 960
a A C G G K L S N W E P K D D A M S E H R -
961 AGGCATTTTCCCAACTGTCCATTTTGGGAAAATTCTCTAGAAACTCTGAGGTTTAGCATT 1020
a R H F P N C P F L E N S L E T L R F S I -
1021 TCAAATCTGAGCATGCAGACACATGCAGCTCGAATGAGAACATTATGTACTGGCCATCT 1080
a S N L S M Q T H A A R M R T F M Y W P S -

FIG. 3 (PAGE 3 OF 7)

19/61

HUMAN hiap-2

1081	AGTGTTCCAGTTTCAGCCTTGAGCAGCTTGCAAGTGCTGGTTTTTATTATTATGTGGTTCGCAAT	1140
a	S V P V Q P E Q L A S A G F Y Y V G R N	-
1141	GATGATGTCAAATGCTTTGGTTGTGATGGTGGCTTGAGGTGTTGGGAATCTGGAGATGAT	1200
a	D D V K C F G C D G G L R C W E S G D D	-
1201	CCATGGGTAGAACATGCCAAGTGGTTTCCAAGGTGTGAGTCTTGATACGAATGAAAGGC	1260
a	P W V E H A K W F P R C E F L I R M K G	-
1261	CAAGAGTTTGTGATGAGATTCAAGGTAGATATCCTCATCTTCTTGAACAGCTGTTGTCA	1320
a	Q E F V D E I Q G R Y P H L L E Q L L S	-
1321	ACTTCAGATACCACTGGAGAAGAAATGCTGACCCACCAATTATTCATTTTGGACCTGGA	1380
a	T S D T T G E E N A D P P I I H F G P G	-
1381	GAAAGTCTCTCAGAAAGATGCTGTGTCATGATGAATACACCTGTGGTTAAATCTGCCTTGGA	1440
a	E S S S E D A V M M N T P V V K S A L E	-

FIG. 3 (PAGE 4 OF 7)

20/61

HUMAN hiap-2

1441 ATGGGCTTTAATAGAGACCTGGTGAAACAACAGTTCTAAGTAAATCCTGCAACTGGA 1500
M G F N R D L V K Q T V L S K I L T T G -

1501 GAGAACTATAAACAGTTAATGATATTGTGTCAGCACTTCTTAATGCTGAAGATGAAAAA 1560
E N Y K T V N D I V S A L L N A E D E K -

1561 AGAGAAGAGGAGAGGAAAAACAAGCTGAAGAAATGGCATCAGATGATTTGTCAATTAAT 1620
R E E E K E K Q A E E M A S D D L S L I -

1621 CGGAAGAACAAGATGGCTCTCTTTCAACAATTGACATGTGTGCTTCCTATCCTCGATAAT 1680
R K N R M A L F Q Q L T C V L P I L D N -

1681 CTTTAAAGGCCAATGTAATTAATAACAGGAACATGATATTATAACAAAAACACAG 1740
L L K A N V I N K Q E H D I I K Q K T Q -

1741 ATACCTTTACAAGCGAGAGAACTGATTGATACCATTGGGTTAAGGAAATGCTGGGCC 1800
I P L Q A R E L I D T I W V K G N A A A -

FIG. 3 (PAGE 5 OF 7)

21/61

HUMAN hiap-2

```

1801 AACATCTTCAAAACTGTCTAAAGAAATTGACTCTACATTGTATATAAGAACTTATTGTG
      N I F K N C L K E I D S T L Y K N L F V -
1861 GATAAGAAATGAAGTATATTCCAACAGAAGATGTTTCAGGTCTGTCACCTGGAAGAACA
      D K N M K Y I P T E D V S G L S L E E Q -
1921 TTGAGGAGGTTGCAAGAAGAACGAACTTGTAAGTGTTGATGGACAAAGTTTCTGTT
      L R R L Q E E R T C K V C M D K E V S V -
1981 GTATTATTCCCTTGTTGTCATCTGGTAGTAGTATGCCAGGAATGTGCCCCCTTCTCTAAGAAA
      V F I P C G H L V V C Q E C A P S L R K -
2041 TGCCCTATTGCAGGGGTATAATCAAGGTACTGTTCGTACATTTCTCTTAAAGAAA
      C P I C R G I I K G T V R T F L S *
2101 ATAGTCTATATTTAACCTGCATATAAAGGTCTTTAAAAATATTGTTGAACACACTGAAGCC
      a

```

FIG. 3 (PAGE 6 OF 7)

22/61

HUMAN hiap-2

```

2161 ATCTAAAGTAAAAAGGGAATTATGAGTTTTCCTAATTAGTAACATTCATGTTCTAGTCTGC 2220
      -
      a

2221 TTTGGTACTAATAATCTTGTCTTGAAAGATGGTATCATATATTTAATCTTAATCTGTT 2280
      -
      a

2281 TATTACAAGGGAAGATTATGTTTGGTGAACATATATTAGTATGTGTACCTAAGGG 2340
      -
      a

2341 AGTAGCGTCXCTGCTTATGCATCATTTTCAGGAGTTACTGGATTTGTTGTTCTTCAG 2400
      -
      a

2401 AAAGCTTTGAAXACTAAATTATAGTGTAGAAAAGAACTGGAAACCAGGAACCTGGAGTT 2460
      -
      a

2461 CATCAGAGTTATGGTGCCGGAATTGTCTTTGGTGCTTTTTCACCTGTGTGTTTAAAAATAAGGA 2520
      -
      a

2521 TTTTCTCTTATTCTCCCCCTAGTTTGTGAGAAACATCTCAATAAAGTGCTTTAAAAAG 2580
      -
      a

```

FIG. 3 (PAGE 7 OF 7)

MOUSE xiap

SEQ ID NO:9

1 G A C A C T C T G C T G G C G G C G G C C C C T C C T C C G G A C C T C C C C C T C G G G A A C C G T C G C C C
60

a

61 G C G G C G C T T A G T A G T G C T T G G C G G A A A G G T G A C A A G T C C T A T T T C C A
120

a

121 G A G A A G A T G A C T T T T A A C A G T T T T G A A G G A A C T A G A A C T T T T G T A C T T G C A G A C A C C A A T
180

SEQ ID NO:10 a

23/61
181 M T F N S F E G T R T F V L A D T N
240 A A G G A T G A A G A A T T T G T A G A A G A G T T A A T A G A T T A A A A C A T T T G C T A A C T T C C C A A G T

a

K D E E F V E E F N R L K T F A N F P S
241 A G T A G T C C T G T T C A G C A T C A A C A T T G C G C G A G C T G G G T T C T T T A T A C C G G T G A A G G A
300

a

S S P V S A S T L A R A G F L Y T G E G
301 G A C A C C G T G C A A T G T T T C A G T T G T C A T G C G G C A A T A G A T A G A T G C C A G T A T G G A G A C T C A
360

a

D T V Q C F S C H A A I D R W Q Y G D S

FIG. 4 (PAGE 1 OF 6)

24/61

MOUSE xiap

```

361  GCTGTTGGAAGACACAGGAGATATCCCCAAATTGCAGATTATCAATGGTTTATTTT 420
      A V G R H R R I S P N C R F I N G F Y F -
421  GAAAATGGTGCTGCACAGTCTACAAATCCTGGTATCCAAAATGGCCAGTACAAATCTGAA 480
      E N G A A Q S T N P G I Q N G Q Y K S E -
481  AACTGTGTGGAAATAGAAATCCTTTTGCCCTGACAGGCCACCTGAGACTCATGCTGAT 540
      N C V G N R N P F A P D R P P E T H A D -
541  TATCTCTTGAGAACTGGACAGGTTGTAGATATTTCAGACACCATATACCCGAGGAACCT 600
      Y L L R T G Q V V D I S D T I Y P R N P -
601  GCCATGTGTAGTGAAGAGCCAGATTGAAGTCATTTTCAGAACTGGCCGACTATGCTCAT 660
      A M C S E E A R L K S F Q N W P D Y A H -
661  TTAACCCCCAGAGAGTTAGCTAGTGGCCCTCTACTACACAGGGGCTGATCAAGTG 720
      L T P R E L A S A G L Y Y T G A D D Q V -

```

FIG. 4 (PAGE 2 OF 6)

MOUSE xiap

721 CAATGCTTTTGTGGGGGAAACTGAAAAATTGGGAACCCCTGTGATCGTGCCCTGGTCA 780
a Q C C C C G G K L K N W E P C D R A W S -
781 GAACACAGGAGACACTTCCCAATTGCTTTTGTGTTTGGGCCGGAACGTTAATGTTCTGA 840
a E H R R H F P N C F F V L G R N V N V R -
841 AGTGAATCTGGTGTGAGTTCTGTAGGAATTCCCAAAATTCACAACTCTCCAAGAAAT 900
a S E S G V S S D R N F P N S T N S P R N -
901 CCAGCCATGGCAGAAATAGACACGGATCGTACTTTTGGAAACATGGATATACTCAGTT 960
a P A M A E Y E A R I V T F G T W I Y S V -
961 AACAAAGGAGCAGCTTCCAAGAGCTGGATTATGCTTTAGGTGAAGCGGATAAAGTGAAG 1020
a N K E Q L A R A G F Y A L G E G D K V K -
1021 TGCTTCCACTGTGGAGGAGGGCTCACGGATTGGAAGCCCAAGTGAAGACCCCTGGGACCAG 1080
a C F H C G G G L T D W K P S E D P W D Q -

25/61

FIG. 4 (PAGE 3 OF 6)

26/61

FIG. 4 (PAGE 4 OF 6)

MOUSE xiap

1441 CAGCTAAGCGCCTACAAGAGGAGAAGCTTCCAAAATCTGTATGGATAGAAATATTGCT 1500
a Q L R R L Q E E K L S K I C M D R N I A -
1501 ATCGTTTTTTCCTTGTGGACATCTGGCCACTTGTAACAGTGTGCAGAAAGCAGTTGAC 1560
a I V F F P C G H L A T C K Q C A E A V D -
1561 AAATGTCCCATGTGCTACACCGTCATTACGTTCAACCAAAAATTTTATGCTTAGTGG 1620
a K C P M C Y T V I T F N Q K I F M S * -
1621 GGCACCACATGTTATGTTCTTCTGCTCTAATGAATGTGAATGGAGCGAACTTTAAG 1680
a -
1681 TAATCCTGCATTTGCATTCATTAGCATCCTGCTGTTTCCAAAATGGAGACCAATGCTAAC 1740
a -
1741 AGCACTGTTTCCGCTCTAAACATTCAATTTCTGGATCTTTTCGAGTTATCAGCTGTATCATT 1800
a -

FIG. 4 (PAGE 5 OF 6)

27/61

28/61

FIG. 4 (PAGE 6 OF 6)

29/61

M-hiap-1

SEQ ID NO:39
1 GAATTCGGGAGACCTACACCCCGGAGATCAGAGGTCAATTGCTGGCGTTCAGAGCCTAG + 60
GAAAGTGGCGTGGCGTATCAGCCTAGCAGTAAACCGACCAGAGCCATGCACAAACTAC +
61 ATCCCAGAGAAAGACTGTCCCTTCCCTCCCTGTCACTCTCACCATGAACATGGTTCAA + 120
121 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 180
M N M V Q -

SEQ ID NO:40
181 GACAGCGCCTTCTAGCCAAGCTGATGAAGAGTGCTGACACCTTTTGAGTTGAAGTATGAC + 240
D S A F L A K L M K S A D T F E L K Y D -
241 TTTTCCCTGTGAGCTGTACCGATTGTCCACGTATTACAGCTTTTCCAGGGGAGTTCCTGTG + 300
F S C E L Y R L S T Y S A F P R G V P V -

301 TCAGAAAGGAGTCTGGCTCGTGGCTTTTACTACACTGGTGCCAATGACAAGGTCAAG + 360
S E R S L A R A G F Y Y T G A N D K V K -
361 TGCTTCTGTGGCCTGATGCTAGACAACTGGAACAAGGGACAGTCCCATGGAGAAG + 420
C F C C G L M L D N W K Q G D S P M E K -

FIG. 5 (PAGE 1 OF 6)

30/61

FIG. 5 (PAGE 2 OF 6)

M-hiap-1

```

781  TTTGCGTCCGATGGGAACTGAGCAACTGGGAACGTAAGGATGATGCTATGTCAGAGCAC
      F A C D G K L S N W E R K D D A M S E H - 840
      +-----+-----+-----+-----+-----+-----+
841  CAGAGGCATTTCCCCAGCTGTCCGTTCTTAAAGACTTGGGTCAGTCTGCTTCGAGATAC
      Q R H F P S C P F L K D L G Q S A S R Y - 900
      +-----+-----+-----+-----+-----+-----+
901  ACTGTCTTAACCTGAGCATGCAGACACAGCAGCCCGTATTAGAACATTCTCTAACTGG
      T V S N L S M Q T H A A R I R T F S N W - 960
      +-----+-----+-----+-----+-----+-----+
961  CCTTCTAGTGCAGTTCATTTCCAGGAACTTGCAAGTGGGGCTTTTATTATACAGGA
      P S S A L V H S Q E L A S A G F Y Y T G - 1020
      +-----+-----+-----+-----+-----+-----+
1021 CACAGTGATGATCAAGTGTTCATGCTGTGATGGTGGCTGAGGTGCTGGAATCTGGA
      H S D D V K C L C C D G G L R C W E S G - 1080
      +-----+-----+-----+-----+-----+-----+
1081 GATGACCCCTGGGTGGAACATGCCAAGTGGTTTCCAAGGTGTGAGTACTTGCTCAGAATC
      D D P W V E H A K W F P R C E Y L L R I - 1140
      +-----+-----+-----+-----+-----+-----+
1141 AAAGGCCAAGAATTGTGTCAGCCCAAGTTCAAGCTGGGTATCCTCATCTACTTGAGCAGCTA
      K G Q E F V S Q V Q A G Y P H L L E Q L - 1200
      +-----+-----+-----+-----+-----+-----+

```

31/61

FIG. 5 (PAGE 3 OF 6)

M-hiap-1

1201 TTATCTACGTCCCGAAGATGAGAATGCAGACGCAGCAATCGTGCAATTTGGC
L S T S D S P E D E N A D A A I V H F G - 1260

1261 CCTGGAGAAAGTTCGGAAGATGTCGTCAATGAGCAGCGCCTGTGTTAAAGCAGCCCTG
P G E S S E D V V M M S T P V V K A A L - 1320

1321 GAAATGGGCTTCAGTAGGAGCCTGGTGAGACAGACGGTTCAGTGGCAGATCCTGGCCACT
E M G F S R S L V R Q T V Q W Q I L A T - 1380

1381 GGTGAGAACTACAGGACCGTCAGTGACCTCGTTATAGGCTTACTCGATGCAGAAGACGAG
G E N Y R T V S D L V I G L L D A E D E - 1440

1441 ATGAGAGAGCAGATGGAGCGCGCCGAGGAGGAGTCAATGATCTAGCACTA
M R E E Q M E Q A A E E E E S D D L A L - 1500

1501 ATCCGGAAGAACAAAATGGTGCTTTTCCCAACATTTGACGTGTGTGACACCAATGCTGTAT
I R K N K M V L F Q H L T C V T P M L Y - 1560

32/61

FIG. 5 (PAGE 4 OF 6)

33/61

M-hiap-1

```

1561  TGCCTCCTAAGTGAAGGCCATCACTGAACAGGAGTGCAATGCTGTGAAACAGAAACCA
      C L L S A R A I T E Q E C N A V K Q K P - 1620
1621  CACACCTTACAAGCAAGCACACTGATTGATACTGTGTAGCAAAAGGAAACACTGCAGCA
      H T L Q A S T L I D T V L A K G N T A A - 1680
1681  ACCTCATTCAGAAACTCCCTTCGGGAAATTGACCCCTGCGTTATACAGAGATATATTGTG
      T S F R N S L R E I D P A L Y R D I F V - 1740
1741  CAACAGGACATTAGGAGTCTTCCCACAGATGACATTGCAGCTCTACCAATGGAAGAACAG
      Q Q D I R S L P T D D I A A L P M E E Q - 1800
1801  TTGCGGCCCTCCCGAGGACAGAAATGTGTAAAGTGTGTATGACCGAGAGGTATCCATC
      L R P L P E D R M C K V C M D R E V S I - 1860
1861  GTGTTTCCTTCCCTGTGGCCATCTGGTCGTGTGCAAGACTGCGCTCCCTCTCTGAGGAAG
      V F I P C G H L V V C K D C A P S L R K - 1920

```

FIG. 5 (PAGE 5 OF 6)

34/61

M-hiap-1

1921	TG	T	C	C	C	A	T	C	T	G	T	A	G	G	C	A	C	A	T	C	A	A	G	G	C	A	C	A	T	T	T	C	T	C	T	G	A	A	C	A	A	G	A										
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
	C	P	I	C	R	G	T	I	K	G	T	V	R	T	F	L	S	*																																			
1981	C	T	A	A	T	G	G	T	C	C	A	T	T	C	A	G	C	C	A	G	G	A	A	G	T	T	C	A	C	T	G	T	C	A	C	T	C	C	C	A	G	T	T	C	C	A	T						
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
2041	T	C	G	G	A	A	C	T	T	G	A	G	G	C	C	A	G	C	C	T	G	G	A	T	A	G	A	A	T	G	A	A	T	G	A	A	T	G	A	A	T	G	A	A	T	G	A	A	T	G	A	A	T
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2101	G	A	A	A	A	C	T	T	T	G	T	C	T	G	A	A	T	G	A	A	T	G	A	A	T	G	A	A	T	G	A	A	T	G	A	A	T	G	A	A	T	G	A	A	T	G	A	A	T	G	A	A	T
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2161	T	T	C	C	T	T	A	A	A	G	T	G	C	T	A	T	T	G	T	T	C	C	C	A	A	C	T	C	A	G	A	A	A	T	T	G	T	T	T	T	T	C	T	G	T	A	A	C	A	T	A		
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2221	T	A	C	T	G	C	A	T	C	T	A	A	A	G	T	A	T	T	C	A	T	A	T	T	C	A	T	A	T	T	C	A	T	A	T	T	C	A	T	A	T	T	C	A	T	A	T	T	C	A	T	A	
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2281	T	G	T	C	T	T	G	T	T	C	C	T	G	A	A	A	G	C	T	G	G	T	T	A	T	C	A	T	C	T	G	A	T	A	T	A	T	C	A	T	A	T	A	T	C	A	T	A	T	A	T	C	
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2341	G	G	C	T	A	G	A	T	C	C	A	T	G	A	A	C	C	A	G	C	T	G	C	A	A	A	G	A	T	C	T	C	A	C	G	T	A	A	A	T	A	A	A	T	A	A	A	A	A	A	A	A	A
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2401	A	G	A	A	C	G	A	A	A	G	G	A	A	T	T	C	C	T	G	T	C	C	A	A	T	G	T	A	T	A	C	T	C	T	T	C	A	G	A	C	T	A	A	T	A	A	T	A	A	T	A	A	
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2461	T	A	T	C	A	A	G	C	C	T	T	C	T	A																																							

FIG. 5 (PAGE 6 OF 6)

35/61

M-hiap-2

```

SEQ ID NO:41  CTGTGGTGAGATCTATTGTCCAAGTGGTGAGAAACTTCACTCTGGAAGTTAAGCGGTCA
1  -----+-----+-----+-----+-----+-----+-----+ 60
GAAATACTATTACTACTCATGGACAAAAGTGTCTCCAGAGACTCGCCCAAGGTACCTTA
61  -----+-----+-----+-----+-----+-----+-----+ 120
CACCCAAAACCTTAAACGTATAATGGAGAAAGAGCACAAATCTTGTCAAAATTGGACAAAGGA
121 -----+-----+-----+-----+-----+-----+-----+ 180
SEQ ID NO:42  M E K S T I L S N W T K E -
GAGCGAAGAAAAATGAAGTTGACTTTTCGTGTGAACTCTACCGAATGTCTACATATTC
181 -----+-----+-----+-----+-----+-----+-----+ 240
S E E K M K F D F S C E L Y R M S T Y S -
AGCTTTTCCAGGGAGTTCCTGTCTCAGAGAGAGTCTGGCTCGTGGCTTTTATTA
241 -----+-----+-----+-----+-----+-----+-----+ 300
A F P R G V P V S E R S L A R A G F Y Y -
TACAGGTGTGAATGACAAAAGTCAAGTGCTTCTGTGTGGCCTGATGTTGGATAACTGGAA
301 -----+-----+-----+-----+-----+-----+-----+ 360
T G V N D K V K C F C C G L M L D N W K -
ACAAGGGACAGTCCTGTGAAAAGCACAGACAGTTCTATCCCAGCTGCAGCTTTGTACA
361 -----+-----+-----+-----+-----+-----+-----+ 420
Q G D S P V E K H R Q F Y P S C S F V Q -

```

FIG. 6 (PAGE 1 OF 6)

36/61

M-hiap-2

421 GACTCTGCTTTCAGCCAGTCTGCAGTCTCCATCTAAGAATAATGTCTCCTGTGAAAAGTAG + 480
T L L S A S L Q S P S K N M S P V K S R -

481 ATTTGCACATTCTGTCACCTCTGGAACGAGGTGGCATTCACTCCAACCTGTGCTCTAGCCC + 540
F A H S S P L E R G G I H S N L C S S P -

541 TCTTAATTCTAGAGCAGTGGGAAGACTTCTCATCAAGGATGGATCCCTGCAGCTATGCCAT + 600
L N S R A V E D F S S R M D P C S Y A M -

601 GAGTACAGAAGAGGCCAGATTTCTTACTTACAGTATGTGGCCCTTAAGTTTCTGTCACC + 660
S T E E A R F L T Y S M W P L S F L S P -

661 AGCAGAGCTGGCCAGAGCTGGCTTCTATTACATAGGCCCTGGAGACAGGGTGGCCTGTTT + 720
A E L A R A G F Y Y I G P G D R V A C F -

721 TGCCTGTGTGGAAACTGAGCAACTGGGAACCAAGGATTATGCTATGTCAGAGCACCG + 780
A C G G K L S N W E P K D Y A M S E H R -

FIG. 6 (PAGE 2 OF 6)

37/61

M-hiap-2

```

781  CAGACATTTCCCACTGTCCATTCTTGAAATACTTCAGAAACACAGGTTAGTAT
      R H F P H C P F L E N T S E T Q R F S I -
840  +-----+-----+-----+-----+-----+-----+
      ATCAAATCTAAGTATGCAGACACACTCTGCTCGATTGAGGACATTTCTGTACTGGCCACC
841  +-----+-----+-----+-----+-----+-----+
      S N L S M Q T H S A R L R T F L Y W P P -
900  +-----+-----+-----+-----+-----+-----+
      TAGTGTTCCTGTTCAGCCCGAGCAGCTTGCAAGTCTGGATTCTATTACGTGGATCGCAA
901  +-----+-----+-----+-----+-----+-----+
      S V P V Q P E Q L A S A G F Y Y V D R N -
960  +-----+-----+-----+-----+-----+-----+
      TGATGATGTC AAGTGCCTTGTGTGATGGTGGCTTGAGATGTTGGGAACCTGGAGATGA
961  +-----+-----+-----+-----+-----+-----+
      D D V K C L C C D G G L R C W E P G D D -
1020 +-----+-----+-----+-----+-----+-----+
      CCCCTGGATAGAACACGCCAAATGGTTTCC AAGGTGTAGTCTTGATACGGATGAAGGG
1021 +-----+-----+-----+-----+-----+-----+
      P W I E H A K W F P R C E F L I R M K G -
1080 +-----+-----+-----+-----+-----+-----+
      TCAGGAGTTTGTGTGATGAGATTCAAGCTAGATATCCTCATCTTCTTGAGCAGCTGTTGTC
1081 +-----+-----+-----+-----+-----+-----+
      Q E F V D E I Q A R Y P H L L E Q L L S -

```

FIG. 6 (PAGE 3 OF 6)

38/61

M-hiap-2

```

1141  CACTTCAGACACCCAGGAGAAAGAAATGCTGACCCCTACAGAGACAGTGGTGCATTTTGG
      +-----+-----+-----+-----+-----+-----+
      T S D T P G E E N A D P T E T V V H F G - 1200
1201  CCCTGGAGAAAGTTCGAAAGATGTCGTATGATGAGCACGCCCTGTGGTTAAAGCAGCCCTT
      +-----+-----+-----+-----+-----+-----+
      P G E S S K D V V M M S T P V V K A A L - 1260
1261  GGAATGGGCTTCAGTAGAGCCTGGTGAGACAGCGGTTCAGCGGCAGATCCTGCCAC
      +-----+-----+-----+-----+-----+-----+
      E M G F S R S L V R Q T V Q R Q I L A T - 1320
1321  TGGTGAGAACTACAGACCGCTCAATGATATTGTCTCAGTACTTTTGAATGCTGAAGATGA
      +-----+-----+-----+-----+-----+-----+
      G E N Y R T V N D I V S V L L N A E D E - 1380
1381  GAGAAGAGAGAGGAGAGGAAAGACAGACTGAAGAGATGGCATCAGGTGACTTATCACT
      +-----+-----+-----+-----+-----+-----+
      R R E E E K E R Q T E E M A S G D L S L - 1440
1441  GATTCGGAAGATAGATGGCCCTCTTTCAACAGTTGACACATGTCCTTCCTATCCTGGA
      +-----+-----+-----+-----+-----+-----+
      I R K N R M A L F Q Q Q L T H V L P I L D - 1500

```

FIG. 6 (PAGE 4 OF 6)

M-hiap-2

39/61

```

1501 TAATCTTCTTGAGGCCAGTGTAATTACAAAACAGGAACATGATATTATTAGACAGAAAC
      N L L E A S V I T K Q E H D I I R Q K T - 1560
1561 ACAGATACCCTTACAAGCAAGAGAGCTTATTGACACCGTTTGTAGTCAAGGAAATGCTGC
      Q I P L Q A R E L I D T V L V K G N A A - 1620
1621 AGCCAACATCTTCAAAAACCTCTCTGAAGGAATTGACTCCAGTTATATGAAAACTTATT
      A N I F K N S L K G I D S T L Y E N L F - 1680
1681 TGTGAAAAGAATATGAAGTATATTCCAACAGAACGTTTCAGGCTTGTCATTGGAAGA
      V E K N M K Y I P T E D V S G L S L E E - 1740
1741 GCAGTGCGGAGATTACAAGAAGAACGAACTTGCAAAGTGTGTATGGACAGAGAGGTTTC
      Q L R R L Q E E R T C K V C M D R E V S - 1800
1801 TATTGTGTTTCATTCGTTGGTCACTAGTAGTCTGCCAGGAATGTGCCCTTCTCTAAG
      I V F I P C G H L V V C Q E C A P S L R - 1860

```

FIG. 6 (PAGE 5 OF 6)

M-hiap-2

40/61

1861	GAAGTCCCCCATCTGCAGGGGACAAATCAAGGGACTGTGCGCACATTTCTCTCATGAGT	1920
	K C P I C R G T I K G T V R T F L S *	
1921	GAAGATGGTCTGAAAGTATTGTTGGACATCAGAAGCTGTGAGAACAAAGAATGAACATAC	1980
1981	TGATTTTCAGCTCTTCAGCAGGACATTTCTACTCTCTTCAAGATTAGTAATCTTGCTTTAT	2040
2041	GAAGGTAGCATTGATATTAAAGCTTAGTCTGTGCAAGGGAAGGTCTATGCTGTTGAG	2100
2101	CTACAGGACTGTCTGTTCCAGAGCAGGAGTTGGGATGCTTGCTGTATGTCCTTCAGGA	2160
2161	CTTCTTGGGATTTGGGAATTGGGGAAAGCTTTGGAATCCAGTGATGTGGAGCTCAGAAA	2220
2221	TCCTGGAACCCAGTGACTCTGGTACTCAGTAGATAGGTACCCCTGTACTTCTTGGTGCTTT	2280
2281	TCCAGTCTGGGAAATAAGGAGGAATCTGCTGCTGGTAAAAATTTGCTGGATGTGAGAAAT	2340
2341	AGATGAAAAGTGTTCCGGGTGGGGCGGTGCATCAGTGTAGTGTGTCAGGGATGTATGCAG	2400
2401	GCCAAACACTGTGTAG	
	-----+----- 2416	

FIG. 6 (PAGE 6 OF 6)

41/61

Alignment of BIR (Baculoviral IAP Repeats) Domains

Baculovirus	
Cp_iap	Cydia pomonella
Op_iap	Orgyia pseudotsugata
Human	
xiap	IAP on X chromosome
hiap1, hiap2	two different human IAP genes
Mouse	
m-xiap	mouse homologue of human xiap gene
Insect	
diap	Drosophila IAP gene, not clearly a homologue of xiap or hiap

FIG. 7

note on consensus: The consensus line represents amino acids or very similar amino acids which are present in 14 of the 19 BIR sequences at each position. Capitalized residues are those that are in the consensus sequence.

SEQ ID NO:11	Op_iap-1	1	kaarLgTYtn	WPvqf.l	lepa	rMaagGFIYI	GrGDeVrCaf	CkveItmWv	gdDpeIdHr	waPqCpFV	68
SEQ ID NO:14	Cp_iap-1		eevRLaTFek	WPvaf.l	lepe	tMaagGFIYI	GrSDeVrCaf	CkveImrWke	gdDpaadHrk	waPqCpFV	
SEQ ID NO:15	diap-2		eanRLvTFkd	WPvnp.l	ltpq	aLaAGFIYI	nrLDhVrCv	CngvIakWek	nDnafeeHr	zfPqCpFV	
SEQ ID NO:16	m-xiap-1		efnRLkTFan	FPagspvsa	tlarAGFLYt	GegDtVrCfs	ChaaIdrWqy	gDsaavgrHrk	vspnCrFI		
SEQ ID NO:17	xiap-1		efnRLkTFan	FPagspvsa	tlarAGFLYt	GegDtVrCfs	ChaaIdrWqy	gDsaavgrHrk	vspnCrFI		
SEQ ID NO:18	hiap1-1		elyRMaTYst	FPagvpsae	slarAGFIYt	GvndKvKcFh	CglmIdnWkl	gDapIqkHtk	lyPscCrFI		
SEQ ID NO:19	hiap2-1		elyRMaTYst	FPagvpsae	slarAGFIYt	GvndKvKcFh	CglmIdnWkl	gDapIqkHtk	lyPscCrFI		
SEQ ID NO:20	m-xiap-2		eearLksFqn	WPdyahltpr	eLaAGLYt	GadDqVqCfc	CggklknWep	cDraawseHr	hfpnCrFI		
SEQ ID NO:21	xiap-2		eearLksFqn	WPdyahltpr	eLaAGLYt	GadDqVqCfc	CggklknWep	cDraawseHr	hfpnCrFI		
SEQ ID NO:22	hiap1-3		eearLkTFtm	WP.lLflapt	dLaAGFIYI	GpgDrVaCfa	CggklknWep	kDnaamseHr	hfpnCrFI		
SEQ ID NO:23	hiap2-3		eearLkTFtm	WP.lLflaps	eLaAGFIYI	GpgDrVaCfa	CggklknWep	kDnaamseHr	hfpnCrFI		
SEQ ID NO:24	m-xiap-3		yearLlTFgt	Wiyav..nke	qlaAGFIYal	GegDkVrCfh	CgggltdWkp	sEdpweqHak	cypGckYl		
SEQ ID NO:25	xiap-3		yearLlTFgt	Wiyav..nke	qlaAGFIYal	GegDkVrCfh	CgggltdWkp	sEdpweqHak	cypGckYl		
SEQ ID NO:26	hiap1-3		haaRkTFfn	WPssvlvtp	qlaAGFIYv	GnsdVrKcFh	CdggllrcWes	gdDpwwqHak	wfPrCefl		
SEQ ID NO:27	hiap2-3		haaRkTFfn	WPssvlvtp	qlaAGFIYv	GnsdVrKcFh	CdggllrcWes	gdDpwwqHak	wfPrCefl		
SEQ ID NO:28	Op_iap-2		eearLkTFae	WPrglkqrpe	eLaAGFIYt	GggDktrCfc	CdggllkdWep	dbapwqHak	wydrCefv		
SEQ ID NO:29	Cp_iap-2		eearLkTFae	WPrglkqrpe	eLaAGFIYt	GggDktrCfc	CdggllkdWep	dbapwqHak	wydrCefv		
SEQ ID NO:30	diap-3		eearLkTFae	WPrglkqrpe	eLaAGFIYt	GggDktrCfc	CdggllkdWep	dbapwqHak	wydrCefv		
SEQ ID NO:31	diap-1		eevRLaTFge	WPlnapvsa	dlvaAGFIY	G-D-V-CF-	C-----W--	-D-----H--	--P-C-FV		
	Cons naus		---RL-TF--	WP-----	-LA-AGFIY-	G-D-V-CF-	C-----W--	-D-----H--	--P-C-FV		

42/61

FIG. 8 (PAGE 1 OF 5)

43/61

151
cp-iapmSD lrl..... EEVRLnTF ekWPv.sfls 200
diap esDnegnsvv dspescscpd lll..... EanRLvTF kdWpn.pnit
m-xiap ppEthadyll rtgqvDiSD tiyprnp.am cSEEARLksF qnWPdyahLt
xiap psEthadyll rtgqvDiSD tiyprnp.am yCEEARLksF qnWPdyahLt
hiap1 anq..... Efsa lmrssypcpM nnEnARLlTF qtWP.ltfLs
hiap2 avE..... Diss srtnpysyam stEEARFlTY hmWP.ltfLs
consensus --E-----D-SD -----M --EEARL-TF --WP---L-

BIR 2

201
cp-iap PetMAknGFY YlGrSDeVrC afCkveimrW kegEdpaaDH kkwPqCPFV 250
diap PqaLAKAGFY YlnrlDhVkc vWCnGviakW EknDnAfeEH krFFPqCPrV
m-xiap PrELAsAGLY YtGadDqVqC FcCGGKLkNW EPcDrAwSEH rRHFPnCfFV
xiap PrELAsAGLY YtGigDqVqC FcCGGKLkNW EPcDrAwSEH rRHFPnCfFV
hiap1 PtDLARAGFY YiGpgDrVaC FaCGGKLsNW EPkDnAmSEH lRHFPkCPFI
hiap2 PsELARAGFY YiGpgDrVaC FaCGGKLsNW EPkDdAmSEH rRHFPnCpFl
consensus P-ELA-AGFY Y-G--D-V-C F-CGGKL-NW EP-D-A-SEH -RHFP-CPFV

251
cp-iap kgidvcgsiv ttnniqnttt hdtiigPahP kyAheaARvk sFhnWPrcmk 300
diap qmgplie.fa tgknldelgi qpttl.Plrp kyAcvdARlr TFtdWPiSni
m-xiap lgrnvnvrse s.gvssdrnF pnStnsPrnP aMAeyeARiv TFgtWiys..
xiap lgrnlnirse sdavssdrnF pnStnlPrnP sMAdyeARif TFgtWiys..
hiap1 enqlqdtSY tvS.....Nl sMqthaARfk TFfnWPSSvl
hiap2 ensl.etlrf siS.....Nl sMqthaARmr TFmyWPSSvp
consensus -----F --S---P-NP -MA---AR-- TF--WP-S--

BIR 3

FIG. 8 (PAGE 2 OF 5)

44/61

BIR 3		301	350
cp-iap	qrpeQMaDAG	FFYtGyGDnt	KCFyCdGGLk dwepeDvPWe QHvrWFdrCa
diap	qpasaLAqAG	LYYqkiGDqV	rCFhCniGLr swqkeDEPwf eHAKWsPkCq
m-xiap	VnkeQLARAG	FYalGeGDkV	KCFhCgGGLt dwkpsEDPWd QHAKcYPgCk
xiap	VnkeQLARAG	FYalGeGDkV	KCFhCgGGLt dwkpsEDPWe QHAKWYPgCk
hiap1	VnpEQLAsAG	FYYvGnsDdV	KCFcCdGGLr cWesgDDPWv QHAKWFFPrCe
hiap2	VqpEQLAsAG	FYYvGrnddV	KCFgCdGGLr cWesgDDPWv eHAKWFFPrCe
consensus	V--EQLA-AG	FYY-G-GD-V	KCF-C-GGL- -W---DDPW- QHAKWFF-C-
		351	400
cp-iap	YvqlvKGrDY	VqkVit.....e.....
diap	FvllakGpay	VseVlattaa	nassqpaTap aptlq.....
m-xiap	YLldeKGQeY	Innihlthp.	LeEslgrTae kt.....Ppltk
xiap	YLleqKGQeY	Innihlths.	LeEclvrTtE kt.....Psltr
hiap1	YLlriKGQeF	IrqVqasyph	LlEqLlTsD spgdenaess iihlePgedh
hiap2	FLlrmKGQeF	VdeIqgryph	LlEqLlTsD ttgeenadpp iihfgPgess
consensus	YL---KGQeY	-----L-E-L--T--	-----P-----
		401	450
cp-iap	..acVLpge.
diap	..adVLmdea	pakeAltLGi	dggvVrnaig rKllssGcaF stldeLlhDi
m-xiap	kiDdtifqnP	mVqeAirMGF	sfkdIKktme eKIqtsGssY lslevLIaDL
xiap	riDdtifqnP	mVqeAirMGF	sfkdIKkime eKIqisGsnY kslevLVaDL
hiap1	seDaIMmntP	vInaAveMGF	srsLVKqtvq rKilatGenY rlvndLVlDL
hiap2	seDaVMmntP	vVksAleMGF	nrdLVKqtv1 sKIlttGenY ktvndiVsaL
consensus	--D-V----P	-V--A--MGF	----VK----- -KI---G--Y -----LV-DL

FIG. 8 (PAGE 3 OF 5)

45/61

451		500
cp-iap
diap	fddagagaal	Evreppe.....
m-xiap	vsAqkDnted	E.....
xiap	vnAqkDsmqD	E.....
hiap1	lnAedEireE	Ererateeke sndlllirkn rmalfqhltc vipildsllt
hiap2	lnAedEkreE	Ekekqaema sddslirkn rmalfqqltc vlpildnllk
consensus	--A-----	E-----
501		550
cp-iap
diap	nttvstaa pvsepipe.....
m-xiap	psapfie pcqattskaa svpipvads i pakpqaaeav
xiap	ssQtsL Q.....
hiap1	ssQtsL Q.....
hiap2	agiineqehd	vikqtQtsL Qarelidtil vkgniaatvf rns lqaeav
consensus	anvinkqehd	iikqtQipl Qarelidtiw vkgnaaanif knclkeidst
	-----	Q--L Q-----

FIG. 8 (PAGE 4 OF 5)

46/61

Ring Zinc Finger		600
551		
cp-iap	...tki....	...Ekep q veDskLCKIC yveEciVcFV
diap	sniskitdei	qkmsvstpng nlsIEEenRq LkDarLCKVC LDeEVgVVFl
m-xiapk diStEEQLRR LqEEkLsKIC MDrnIaIVFf
xiapk eiStEEQLRR LqEEkLCKIC MDrnIaIVFV
hiap1	lyehlfvqqd	ikyiptedvs dlpvEEQLRR LpEErtCKVC MDkEVsIVFI
hiap2	lyknlfvdkn	mkyiptedvs glsIEEQLRR LqEErtCKVC MDkEVsVVFI
consensus	-----	--S-EEQLRR L-EE-LCK-C MD-EV--VF-
601		
cp-iap	PCGHvVaCak	CALSVdKCPM CRkIVtSVlk vYFS.
diap	PCGHLatCnq	CAPsVanCPM CRadIkqfvr tFLS*
m-xiap	PCGHLatCkq	CAeaVdKCPM CytVItfnqk iFMS*
xiap	PCGHLVtCkq	CAeaVdKCPM CytVItfkqk iFMS*
hiap1	PCGHLVvCkd	CAPslrKCPi CRstIkgtvr tFLS*
hiap2	PCGHLVvCqe	CAPslrKCPi CRgIIkgtvr tFLS.
consensus	PCGHLV-C--	CA-SV-KCPM CR-I----- -FLS-

FIG. 8 (PAGE 5 OF 5)

47/61

SEQ ID	NO:32	hiap2	1	46
SEQ ID NO:32	hiap2	EqlrrlqEer	tCKVCMdkEv	svvFlPCGH1
SEQ ID NO:33	hiap1	EqltrlpEer	tCKVCMdkEv	svvFlPCGH1
SEQ ID NO:34	m-xiap	EqltrlqEek	lsKICMdrnl	aiVfFPCGH1
SEQ ID NO:35	xiap	EqltrlqEek	lCKICMdrnl	aiVfVPCGH1
SEQ ID NO:36	diap	EenrglkDar	lCKVCLdeev	gvvFlPCGH1
SEQ ID NO:37	Cp_iap	EkepqqveDsk	lCKICyveec	ivcFvPCGHV
SEQ ID NO:38	Op_iap	aveaeavaDdr	lCKICLgack	tvcFvPCGHV
Consensus		E-----E--	-CKICM----	-V-F-PCGH-
				--C--CA---
				--CP-C

51/61

INCUBATION: OVERNIGHT

S: STANDARDS

	Hg			CEM-CM ₃			GT/CEM			JKT		
HIV	-	-	+	-	-	+	-	-	+	-	-	+
PHA/PMA	-	+	-	-	+	-	-	+	-	-	+	-
S	+	-	-	+	-	-	+	-	-	+	-	-

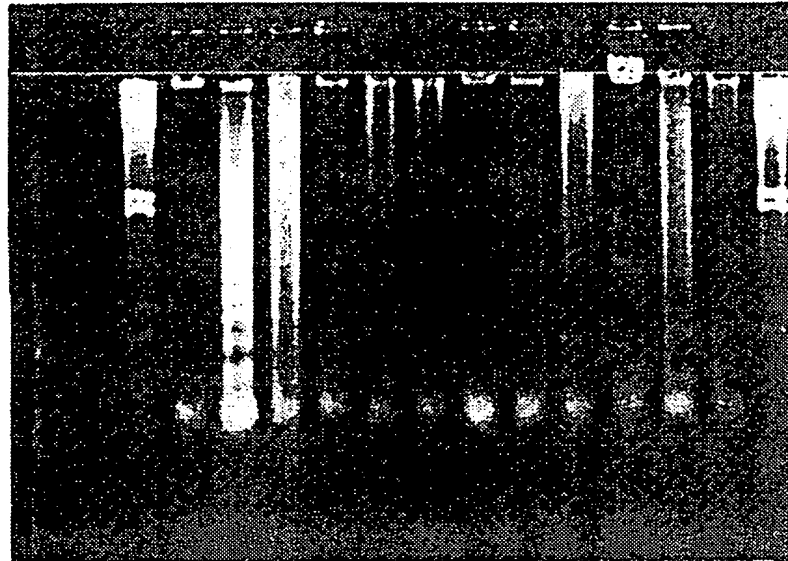
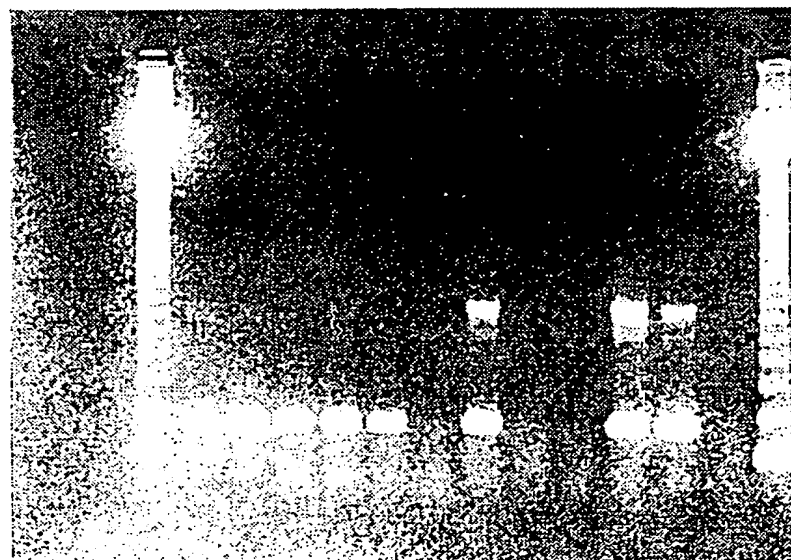


FIG.13A

M | A | B | C | D | M
1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 |



hiap 2a
← hiap 2b
← hiap 1

FIG.13B

52/61

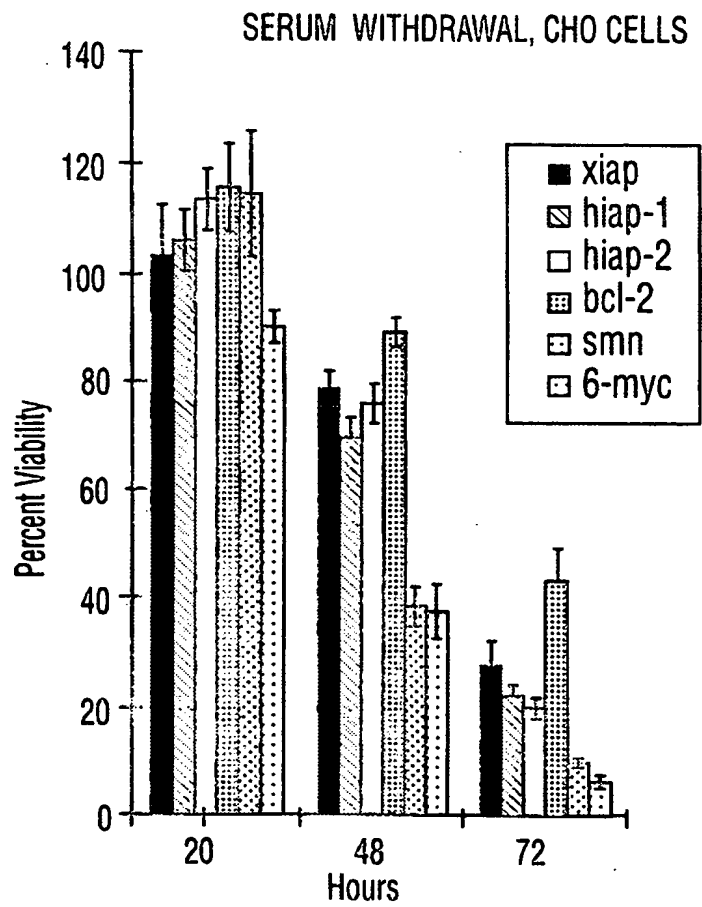


FIG.14A

MENADIONE (20 μ M), CHO Cells. 24hr SURVIVAL

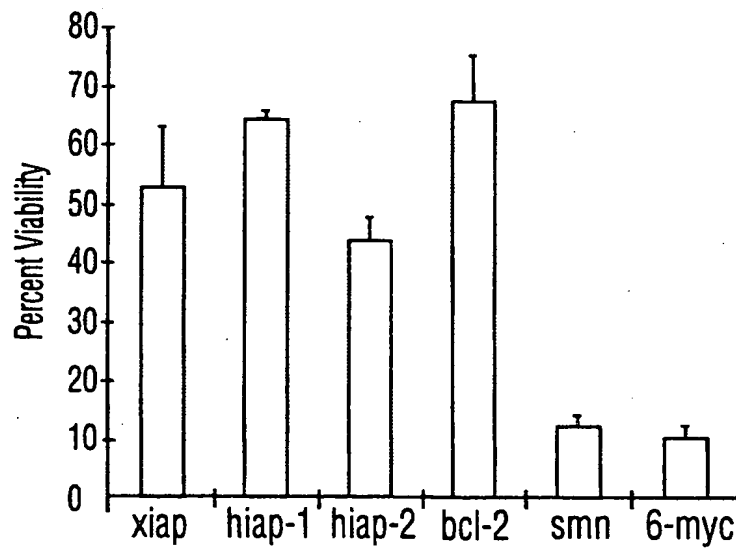


FIG.14B

53/61

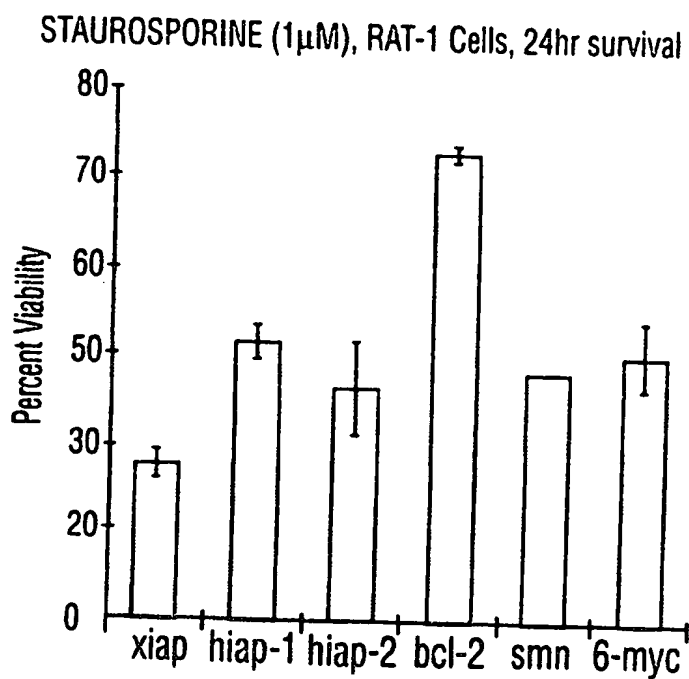
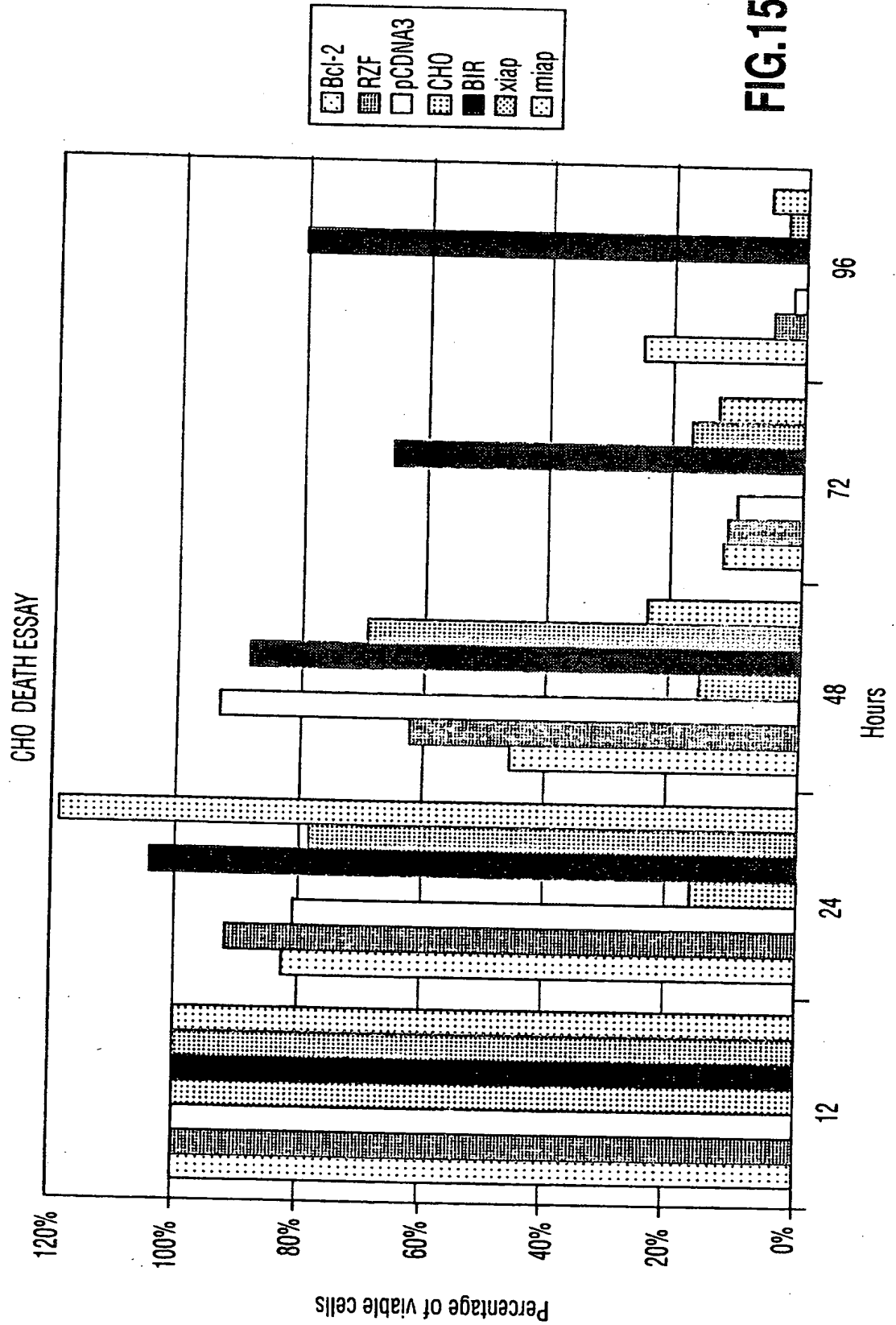


FIG.14C

54/61

FIG.15A



55/61

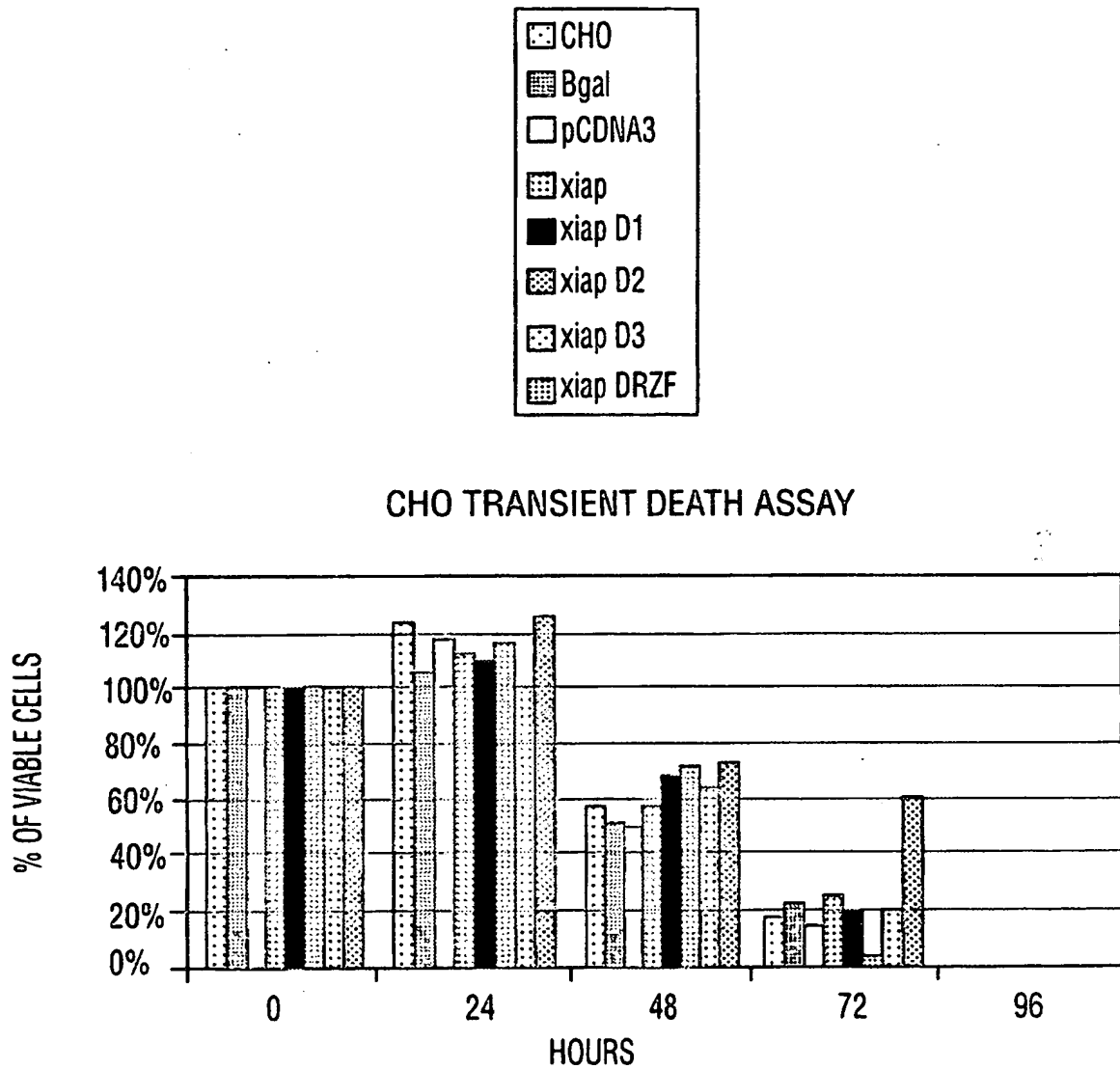


FIG.15B

56/61

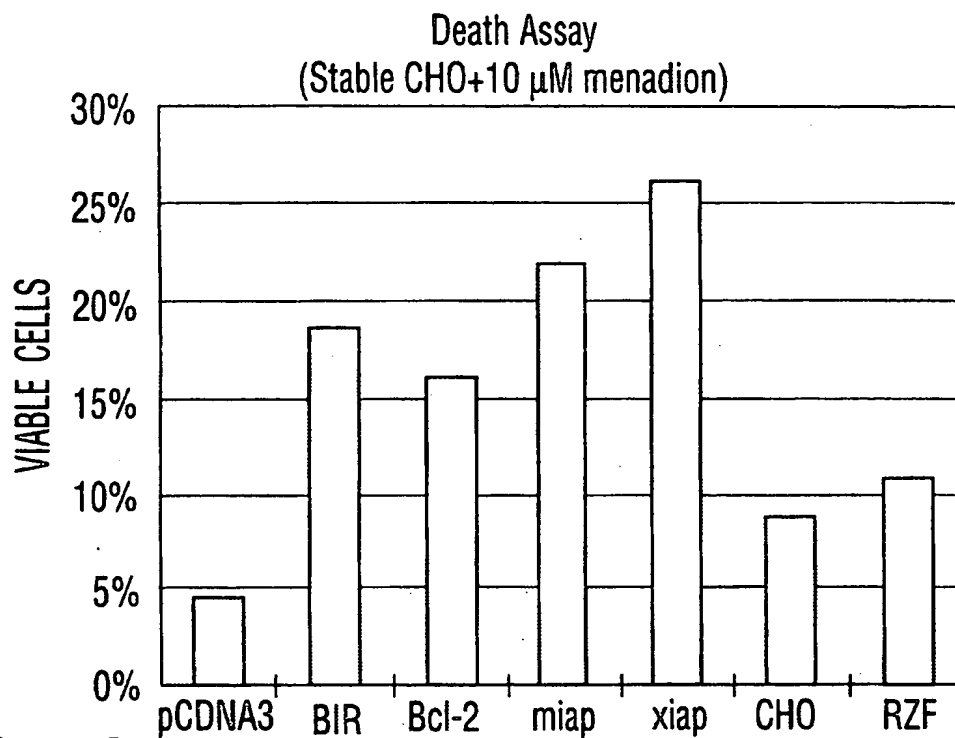


FIG.16A

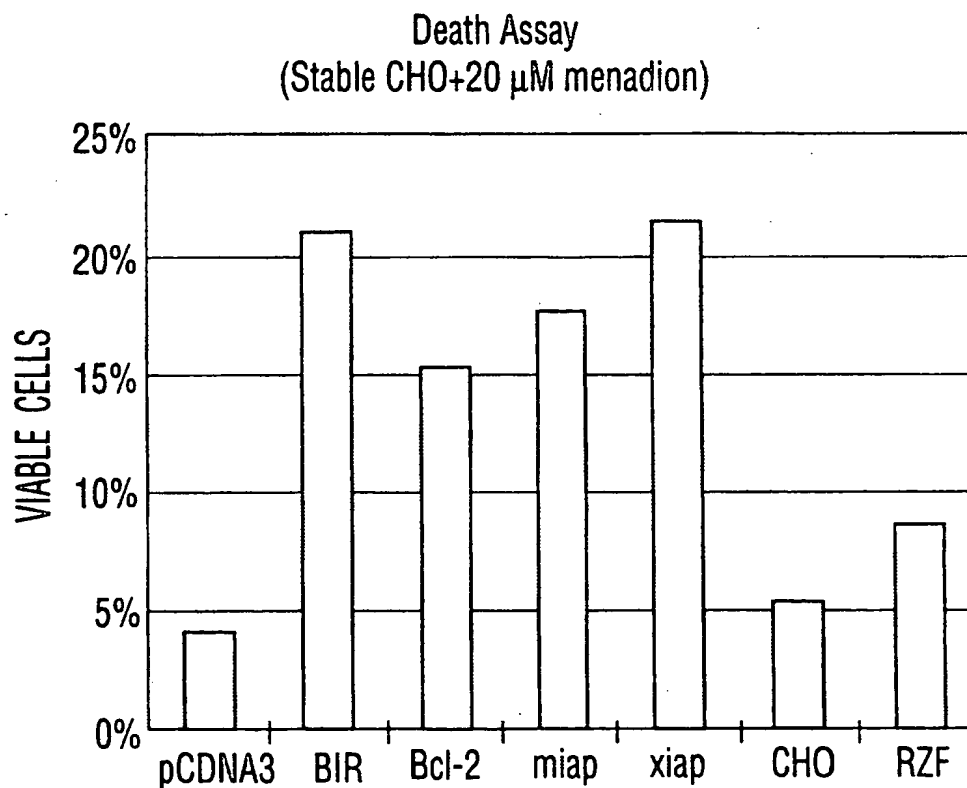


FIG.16B

57/61

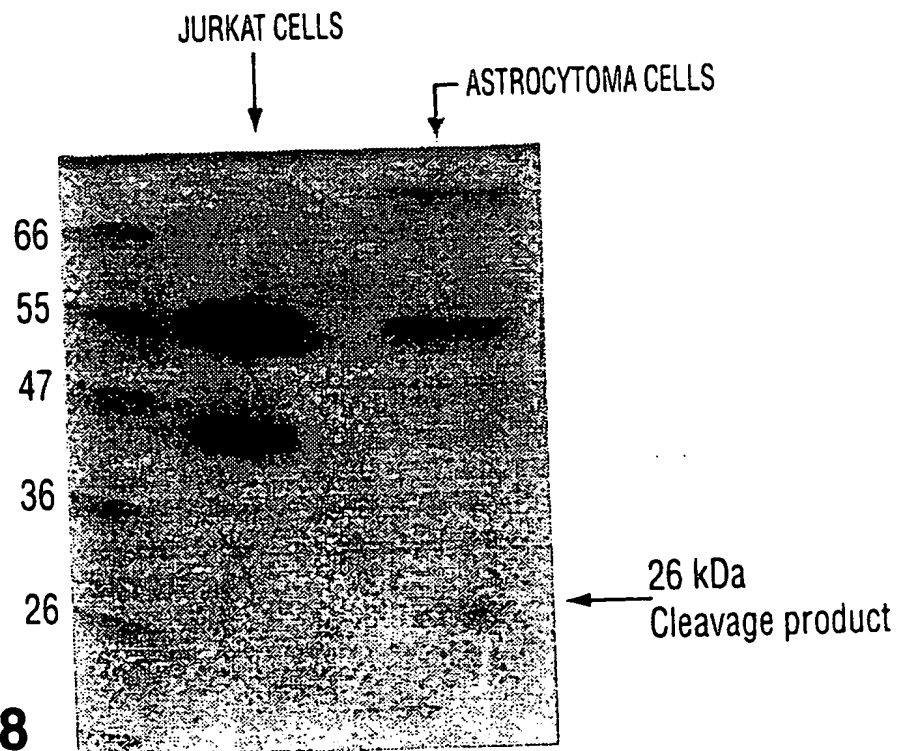


FIG. 18

58/61

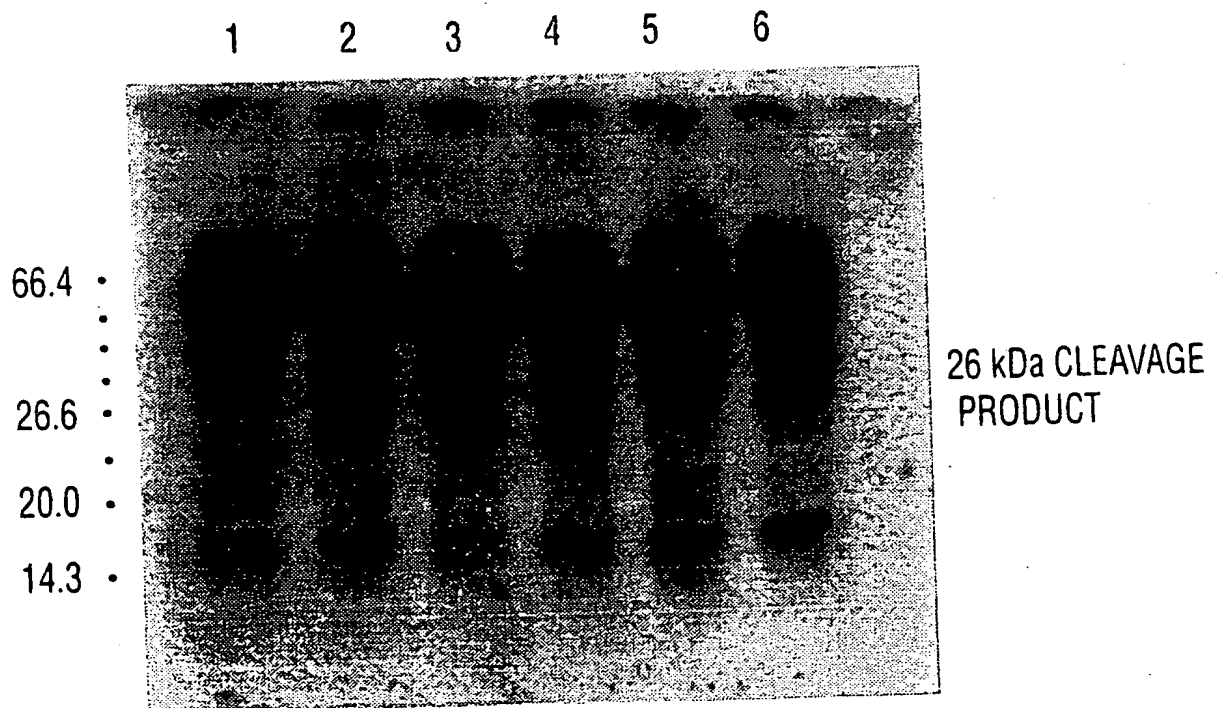


FIG.20

60/61

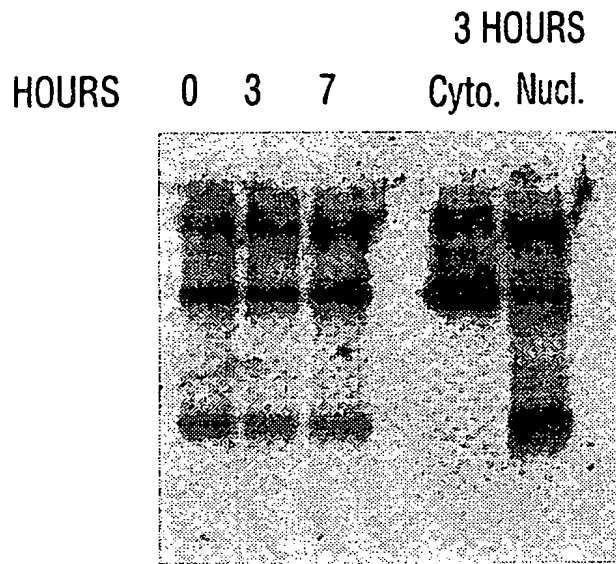


FIG.22A

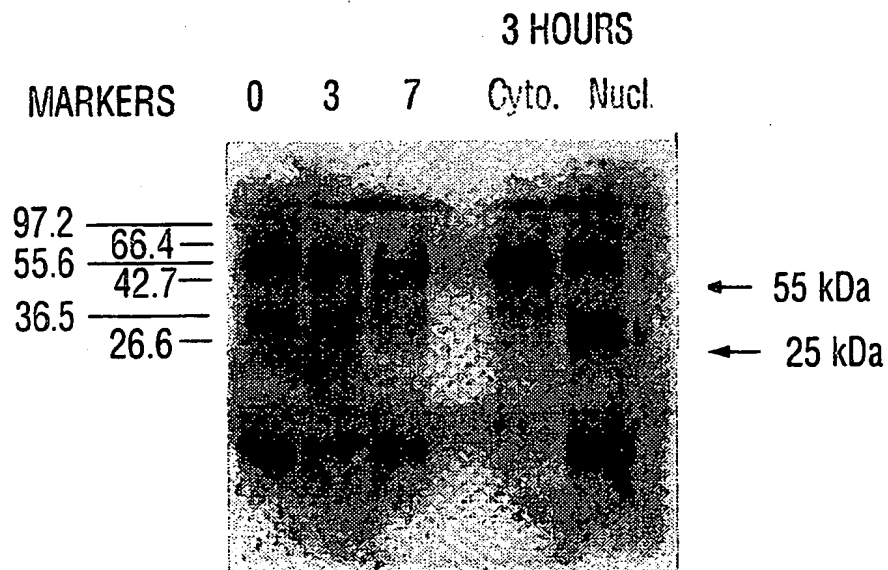


FIG.22B



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08/576,956	22 December 1995 (22.12.95)	US

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Filed on	4 August 1995 (04.08.95)
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(71) Applicant (for all designated States except US): UNIVERSITY OF OTTAWA [CA/CA]; 550 Cumberland, Ottawa, Ontario K1N 6N5 (CA).

(72) Inventors; and

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(74) Agent: MORROW, Joy, D.; Smart & Biggar, 900 - 55 Metcalfe Street, P.O. Box 2999, Station D, Ottawa, Ontario K1P 5Y6 (CA).

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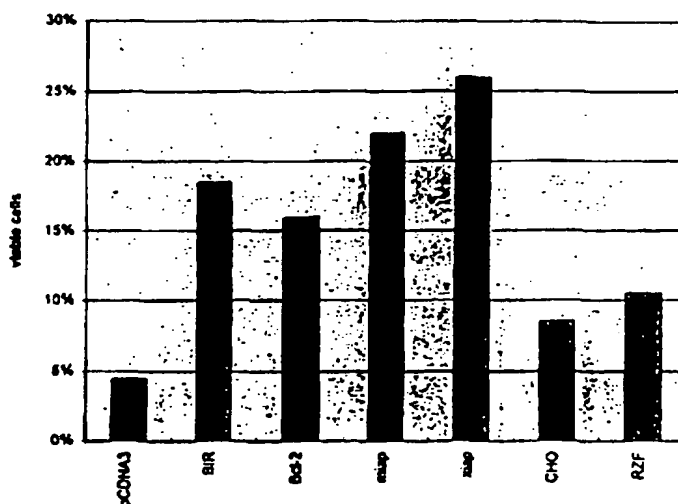
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

With a request for rectification under Rule 91.1(f).

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30 October 1997 (30.10.97)

(54) Title: MAMMALIAN APOPTOSIS INHIBITOR PROTEIN GENE FAMILY, PRIMERS, PROBES AND DETECTION METHODS

BIR = BACULOVIRUS IAP REPEAT
RZF = RING ZINC FINGER

(57) Abstract

Disclosed is substantially pure DNA encoding mammalian IAP polypeptides; substantially pure polypeptides; and methods of using such DNA to express the IAP polypeptides in cells and animals to inhibit apoptosis. Also disclosed are conserved regions characteristic of the IAP family and primers and probes for the identification and isolation of additional IAP genes. In addition, methods for treating diseases and disorders involving apoptosis are provided.

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/IB 96/01022

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 C12N5/10 A01K67/027 A61K38/17
C12Q1/68 C07K16/18 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLEM RJ ET AL: "Control of programmed cell death by the baculovirus genes p35 and iap." MOL CELL BIOL, AUG 1994, 14 (8) P5212-22, UNITED STATES, XP000611843	1-3, 17-19, 22,23, 91,92
Y	cited in the application see the whole document	9-16, 24-38, 49-85, 88,90, 93,94
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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Date of the actual completion of the international search

5 June 1997

Date of mailing of the international search report

17.09.97

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Authorized officer

Gurdjian, D

INTERNATIONAL SEARCH REPORT

Int. Application No.

PCT/IB 96/01022

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROY N ET AL: "The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy [see comments]" CELL, JAN 13 1995, 80 (1) P167-78, UNITED STATES, XP002032295 see the whole document	9-16, 25-38, 49-85, 88,90, 93,94
Y	WO 95 19431 A (SCRIPPS RESEARCH INST ;BARBAS CARLOS F III (US); GOTTESFELD JOEL M) 20 July 1995 see claims 1-52	32-38, 49-85, 88,90, 93,94
Y	WO 94 06814 A (GEN HOSPITAL CORP) 31 March 1994 see page 1, paragraph 3; claim 28	24
A	CROOK NE ET AL: "An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif." J VIROL, APR 1993, 67 (4) P2168-74, UNITED STATES, XP000611841 cited in the application see the whole document	9-16, 25-38, 49-85, 88,90, 93,94
P,X	LISTON P ET AL: "Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes." NATURE, JAN 25 1996, 379 (6563) P349-53, ENGLAND, XP002032296 see the whole document	1-4, 9-20, 25-31
P,X	DUCKETT CS ET AL: "A CONSERVED FAMILY OF CELLULAR GENES RELATED TO THE BACULOVIRUS IAP GENE AND ENCODING APOPTOSIS INHIBITORS" EMBO JOURNAL, 1996, 15, 2685-2694, XP002032297 see the whole document	1-4, 9-20, 25-31
P,X	CLEM RJ ET AL: "ANTI-APOPTOTIC GENES OF BACULOVIRUSES" CELL DEATH AND DIFFERENTIATION, 1996, 3, 9-16, XP000611842 see the whole document	1-4, 9-20, 25-31
P,X	ROTHE M ET AL: "The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins." CELL, DEC 29 1995, 83 (7) P1243-52, UNITED STATES, XP002032302 see the whole document	1-4, 9-20, 25-31

INTERNATIONAL SEARCH REPORT

I national application No.

PCT/IB 96/01022

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 39-48 and claims 34-38, 85, 88, 90 partially as far as they concern an in vivo method, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of that composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

subject 1. (see continuation-sheet)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/IB 96/ 01022

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

*1. Claims 2-4 11 12 14-16 27 30 31 40 42 49 52 53 55-57 62-66 75 76 79 , all completely
claims 1 9 10 13 17-26 28 29 32-39 41 43-48 50 51 54 58-61 67-74 77 78 80-85 88 90-94, all
partially*

Apoptosis inhibiting proteins with ring zinc finger domains and with BIR (baculovirus IAP domain repeat) domains , their nucleic acids ,antibodies and antisense nucleic acids ,to be used in transgenic cells and animals containing them, in therapeutic compositions in methods to inhibit apoptosis in vitro ,methods to their identification and diagnosis

2. Claims 5-7 , all completely

*claims 1 9 10 13 17-26 28 29 32-39 41 43-48 50 51 58-61 67-74 77 78 80-85 88 90-94, all
partially*

Apoptosis inhibiting proteins with no ring zinc finger domain and at least one BIR (baculovirus IAP domain repeat) domain , their nucleic acids , antibodies and antisense nucleic acids , to be used in transgenic cells and animals containing them ,in therapeutic compositions in methods to inhibit apoptosis in vitro , methods to their identification and diagnosis .

3. Claims 8 86 87 89 , all completely

*claims 1 9 10 13 17-26 28 29 32-39 41 43-48 50 51 58-61 67-74 77 78 80-85 88 90-94, all
partially*

Apoptosis inhibiting proteins with a ring zinc finger domain and no BIR (baculovirus IAP domain repeat) domain , their nucleic acids ,antibodies , and antisense nucleic acids ,to be used in transgenic cells and animals containing them ,in therapeutic compositions in methods to inhibit apoptosis in vitro , methods to their identification and diagnosis .

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/18 96/01022

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